JCBFM
Journal of Cerebral Blood Flow & Metabolism
2017, Vol. 37(1S) 445–505

DOI: 10.1177/0271678X17695990
journals.sagepub.com/home/jcbfm
SAGE

BRAIN & BRAIN PET 2017
Poster Viewing Session VI

PS06-001

Poster Viewing Session VI

Impairment of neurovascular coupling after subarachnoid hemorrhage *in vivo*

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Abstract

Introduction: Subarachnoid hemorrhage (SAH) induces acute changes in the cerebral microcirculation such as microthrombosis, microvasospasms, and lack of CO_2 reactivity. New findings *ex vivo* suggest that neurovascular coupling (NVC), the process that increases cerebral blood flow upon neuronal activity, may also be impaired after SAH. The aim of the current study was to investigate NVC after SAH *in vivo*.

Methods: C57BL/6 mice were subjected to either sham surgery or SAH by filament perforation. Neurovascular reactivity was tested 24 hours later by either forepaw stimulation or CO_2 inhalation and visualized in pial and parenchymal arterioles by two-photon microscopy. NVC was also investigated *ex vivo* on acute brain slice preparations.

Results: In sham-operated mice, cerebral vessels dilated to about 130% of baseline after CO_2 inhalation or forepaw stimulation. By contrast, SAH resulted in a complete loss of CO_2 reactivity *in vivo*, and pial and parenchymal vessels did not dilate upon neuronal activation either *in vivo* or *ex vivo* - the majority of vessels even constricted by 10–15%.

Conclusions: These findings indicate that after SAH cerebral vessels do not dilate but constrict upon neuronal activation. This process may further aggravate SAH-induced brain damage. Based on previous results showing that NVC is preserved three hours after SAH, the current results demonstrate that the impairment of NVC is progressive and therapeutic efforts need to be initiated as early as possible after SAH.

Keywords

Subarachnoid hemorrhage, Neurovascular coupling, CO_2 reactivity, *in vivo*, *ex vivo*

PS06-002

Poster Viewing Session VI

New insights into brain blood flow regulation during stroke: Role of electrical communication

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Abstract

Arterial networks coordinates blood flow delivery to the brain in accordance with metabolic demand. To change blood flow magnitude, vasoactive stimuli generated in the grey matter must dilate cortical surface vessels

concerted with penetrating arterioles. These coordinated multi-segmental responses require a common signal (i.e. charge) to be shared among neighboring vascular cells. This process is enabled by gap junctions, intercellular pores that coordinate membrane potential, cytosolic [Ca^{2+}] and myosin light chain phosphorylation responses, along the arterial tree. In this study, we sought to understand intercellular communication in the cerebral circulation and its impact on driving physiological (functional hyperemia) and pathophysiological (collateral flow recruitment) responses. Electrical stimuli spread effectively from cell-to-cell in cerebral arteries, isolated from mice and resected human tissue, in an endothelium dependant manner. Compromising gap junctional communication through the genetic deletion of Connexin40 ($Cx40^{-/-}$), attenuated electrical communication and functional hyperemic responses spreading from penetrating arterioles to the cortical surface vessels. Compromised cell-to-cell communication impaired the recruitment of collateral circulation, during and after experimental stroke, reducing brain blood flow and expanding tissue injury. In closing, our findings demonstrate electrical communication to be an integral component of the cerebral circulation; established by gap junctional resistance and linked to the endothelium. Cell to cell communication drives multi-segmental responses in cerebrovascular networks and its impairment compromises the brain's ability to: 1) match blood flow delivery with metabolic demand; and 2) to salvage brain tissue during stroke. As such, the mechanistic significance of cell to cell communication have to be considered; for understanding flow responses in the cerebral circulation and especially while designing therapeutic paradigms that target blood flow augmentation in the brain.

Abstract

Objectives: Transient neurological events (TNEs) frequently occur after revascularization in adult moyamoya disease (MMD). In the present study, we hypothesized that cortical arterial network disruption may be associated with TNE severity following bypass surgery.

Methods: This retrospective study included 76 hemispheres in 45 consecutive adult MMD patients who underwent direct revascularization surgery at our institution. We classified cortical arterial network disruption grade (NDG) into the following four categories based on angiography: NDG 0, $>$ 90% of suprasylvian cortical branches of the middle cerebral artery demonstrated anterograde filling; NDG 1, 50% to 90%; NDG 2, $<$ 50%; NDG 3, none. TNE severity was assigned one of four grades based on symptom duration and clinical features; grade 0, none; grade 1, mild; grade 2, moderate; grade 3, severe. We evaluated multiple clinical characteristics, including NDG, to identify factors that have a significant association with TNE severity.

Results: Of the 73 hemispheres without perioperative ischemic or hemorrhagic complications, the following degrees of TNEs were developed; grade 0, 33%; grade 1, 30%; grade 2, 22%; grade 3, 15%. We determined that NDG and left-side surgery were significantly associated with TNE severity ($P < 0.01$, and $P = 0.04$, respectively). The NDG had excellent interobserver reliability (weighted κ value $= 0.96$). There were no significant correlations between TNE severity and other clinical backgrounds.

Conclusions: NDG is useful for the prediction of severity of TNEs after revascularization. Disturbed bypass flow spreading may lead to the development of TNEs in adult MMD.

PS06-003

Poster Viewing Session VI

Disruption of cortical arterial network is associated with the severity of transient neurological events after direct bypass surgery in adult moyamoya disease

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PS06-004

Poster Viewing Session VI

Investigating the impact of obesity on post-stroke complications

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Abstract

Objectives: The "obesity paradox" describes improved outcome in obese patients post-stroke and has been attributed to increased body mass protecting against post-stroke weight loss. In contrast, pre-clinical stroke studies have demonstrated that obese animals have a poorer outcome after stroke, with increased ischemic damage and mortality. However, these studies are hindered by investigation of post-stroke changes at acute time-points (typically 24–48 h) and limited clinical relevance. This study aimed to establish how obesity impacts long-term recovery post-stroke in mice, assessing multiple outcomes: neurological damage, metabolic changes, sickness behaviour and cognitive decline.

Methods: Focal cerebral ischaemia was induced by middle cerebral artery (MCA) occlusion in mice maintained on a high fat diet (HFD) or a low fat diet (LFD) for 6 months. As it has previously been shown that HFD-fed-mice have greater ischaemic damage, HFD- and LFD-fed-mice were subjected to 20 and 30 min occlusion of the MCA to attain similar infarct volumes. This allowed us to assess the contribution of obesity to stroke outcome independently of initial infarct. Neurological damage was assessed at 48 h and 51 days by MRI. Metabolic changes were assessed using Echo MRI and comprehensive lab animal monitoring system. Depressive behaviours were assessed using burrowing and nest building tests, and memory assessed by Y-maze and novel object recognition tests.

Results: Long term outcomes were independent of initial infarct as HFD and LFD groups had no significant difference between ischemic infarct volumes. HFD- and LFD-fed-mice had a transient reduction in lean mass which recovered within 14 days and a prolonged reduction in adipose tissue. An acute sickness response was observed in both groups through a reduction in burrowing and nesting behaviours, which resolved by day 14.

Conclusions: Stroke had lasting effects on metabolism and cognition, which were measurable in animals up to 60-days post-stroke in both obese and control mice.

PS06-005

Poster Viewing Session VI

Novel substrates of the small vessel disease related HTRA1 protease identified by proteomics

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Abstract

Loss-of-function mutations in the HTRA1 gene encoding the serine protease high temperature requirement A1 have been identified as a cause for both dominant and recessive forms of small vessel disease (SVD). The molecular mechanisms and signaling pathways involved in the development of these disorders are incompletely understood. We applied a high-end label-free quantification mass spectrometry (LC-MS/MS) approach to determine the proteomes of cerebral microvessels isolated from wild-type and HTRA1 knockout mice. Overall we quantified 3.884 proteins ($\geq$2 unique peptides) among them 117 differentially expressed proteins with marked overrepresentation of proteins that were upregulated in HTRA1-deficient animals. Among upregulated proteins were known substrates of HTRA1 such as latent transforming growth factor (TGFβ)-binding proteins and downstream targets including TGFβ. We further identified several novel proteins with established roles in other types of SVD and vascular development. We confirmed enrichment of several of these targets by immunostaining of isolated microvessels and biochemical approaches. Using in-vitro proteolytic cleavage assays we further showed that several of these targets including members of the WNT signaling

pathway are indeed direct substrates of HTRA1. Collectively, our data establish molecular links between different types of SVD and suggest a possible role of dysregulated WNT signaling in HTRA1-related SVD.

PS06-006

Poster Viewing Session VI

MicroRNA-103-1 is involved in the pathogenesis of ischemic brain damage and may represent a stroke peripheral diagnostic marker

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Abstract

Ischemic stroke is a multi-faced pathology that involves gene reprogramming. Among those genes whose expression is influenced by cerebral ischemia can be included the plasmamembrane protein sodium-calcium exchanger-1 (NCX1), that, by controlling Ca^{2+} and Na^{+} fluxes in a bidirectional way across the synaptic plasma membrane, plays a pivotal role in the regulation of ionic homeostasis in physiological and pathophysiological conditions such as brain ischemia. We have recently identified a microRNA (miR-103-1) able to selectively modulate NCX1 expression in the brain during stroke and whose inhibition by anti-mir-103-1,intracerebroventicularly infused in ischemic rats, causes NCX1 upregulation in brain cortex and striatum accompanied by brain damage reduction.

Since it has been established that microRNAs (miRNAs) can be included in vesicles called exosomes and released in the blood, it has been hypothesized a potential use of these non-coding RNAs as biomarkers for neurological disorders.

In this work, we firstly investigated the expression of miRNA-103-1 in the ischemic penumbra of animals subjected to brain ischemia or to the neuroprotective phenomenon called "Remote Postconditioning" (RemPost), a strategy in which a subliminal ischemia applied to a "distant" organ is able to protect the brain from a previous harmful ischemic insult. As expected, the expression of miRNA103-1 increased after harmful brain ischemia and was dramatically reduced after remote postconditioning.

More interestingly, we were able to demonstrate that miR-103-1 may serve as peripheral prognostic/diagnostic marker of stroke, since its expression level in plasma samples of rats subjected to brain ischemia is proportionate to brain ischemic damage.

These results suggest that miRNA103-1 expression is a marker of ischemic brain damage and, more intriguingly, it is released in the blood in a manner proportionate to brain damage, thus representing a good candidate for stroke diagnosis.

PS06-007

Poster Viewing Session VI

Does long-lasting high sodium intake predispose cerebral blood vessels to stroke? Comparison with sodium-dependent hypertension

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Abstract

High sodium intake is a risk factor of cardio-vascular diseases including arterial hypertension and its major complication - stroke. To what extent prolonged high sodium intake *per se* affects cerebral blood vessels is not clear. To answers this, the experiments were performed on Sprague Dawley rats (SD) divided into following groups: SD with sham unilateral nephrectomy fed standard (0.25% Na, SHAMNS) or high sodium (4% Na) diet (SHAMHS) and SD with unilateral nephrectomy fed high sodium (4% Na) diet (UNXHS). Once a week in the course of 28 days the blood pressure was measured noninvasively and plasma sodium concentration was determined. After 28 days MCAs were harvested and the responses to extravascular administration of: endothelin 1 (ET-1: 10^{-10}, 5×10^{-10}, 10^{-9}, 5×10^{-9}, 10^{-8}M), AT1 angiotensin receptor agonist ($AT1_{agon}$: 5×10^{-10}, 10^{-9}, 5×10^{-9}, 10^{-8}M), endothelium-dependent adenosine-5′-trifosphate (ATP: 10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6}M) and nitric oxide synthesis inhibitor (L-NAME, 10^{-5}M) were studied in

the arteriograph chamber. Arterial blood pressure did not differ between SHAMNS and SHAMHS rats whereas in UNXHS rats it started to increase from the 7th day of the diet reaching maximum after 21 days.
The response of the MCA to vasoconstrictors did not differ between the groups. High sodium diet *per se* did not impair MCA response to ATP (SHAMHS vs SHAMNS) whereas in the group UNXHS this response was significantly diminished when compared to SHAM groups. Administration of L-NAME resulted in significantly smaller constriction of the MCA in SHAMHS and UNXHS in comparison with the response observed in SHAMNS.
The results of this study show that: 1) high sodium diet leads to impaired basal release of NO in the rat MCA, 3) loss of the response of the rat MCA to endothelium-dependent vasodilators in sodium-dependent hypertension occurs at the early stage of the disease.

PS06-008

Poster Viewing Session VI

Structure of the brain bioelectric activity in the stroke patients with various types of polymorphism of the genes ACE, eNOS, MTHFR

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Abstract
Aim: Analysis of the structure of brain bioelectric activity in the stroke patients relative their hereditary polymorphism genes.
Subjects and Methods: Altogether 195 patients after an ischemic stroke were included in the study. Their EEG was recorded on the 16-channel electroencephalograph Neurofax EEG-1100K. Gene type determination by the polymorphism was performed using a technique of the polymerase chain reaction. The followings were studied: genes of the angiotensin-transforming enzyme (ACE), endothelial NO-synthase (eNOS), methyl tetrahydrofolate reductase (MTHFR).
Results: In the post-ischemic stroke patients with genotype DD versus genotype II and ID of the ACE gene, the power within a range of alpha - 1, alpha - 2, beta -1 and beta - 2 was statistically lower in two hemispheres.
Patients of the homozygotic bearers of mutant variant 4a gene eNOS, in contrast to the patients with 4b4b genotype, displayed the high level of power within a range of the slow (delta- and theta-) rhythms in all areas of injured hemisphere against a background of low frequency and power of the alpha-rhythm.
In the heterozygotic patients-bearers of polymorphism GA of gene prothrombin, the frequency of alpha-rhythm in all areas of the intact hemisphere was statistically higher.
The patients-bearers of polymorphism TT of the gene MTHFR versus the patients with polymorphism CC showed, respectively, a statistically higher power in the range of delta and theta-rhythms and a statistically lower power of alpha-rhythm in the injured hemisphere,
Conclusions: The phenotypic peculiarities of brain bioelectric activity are characteristic for the patients with polymorphism of gene ACE, eNOS and MTHFR, whereas the most unfavorable constitutional types of EEG are characteristic for the genotypes DD of gene ACE, TT gene MTHFR, 4a4a gene eNOS and gene GA of the prothrombin.

PS06-009

Poster Viewing Session VI

Enhanced therapeutic potential of nano-curcumin against subarachnoid hemorrhage-induced blood-brain barrier disruption through inhibition of inflammatory response and oxidative stress

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Abstract
Objectives: Curcumin and nano-curcumin both exhibit neuroprotective effects in early brain injury (EBI) after experimental subarachnoid hemorrhage (SAH). However, the mechanism that whether curcumin and its nanoparticles affect blood-brain barrier (BBB) following SAH remains unclearly. This study investigated the effect of curcumin and the poly (lactideco-glycolide) encapsulated curcumin nanoparticles (Cur-NPs) on BBB disruption and evaluated the possible mechanism underlying BBB

dysfunction in EBI using the endovascular perforation rats SAH model.
Methods: Animals were divided randomly into six groups: sham-operated group, vehicle-treated SAH group, curcumin-treated SAH group (150 mg/kg, 300 mg/kg); Cur-NPs-treated SAH group (10 mg/kg, 10 mg/kg). Eighteen rats of each group were for measuring mortality rates analysis (at 24 and 48 h), neurological assessment (at 24 h), glutamate concentration in CSF (at 48 h), LDH activity and release of cyochrome c (at 48 h). Six rats of each group were for detecting brain water content and BBB permeability at 24 h. Six rats of Cur-NPs-treated SAH group were for molecular biological and biochemical experiments at 24 h. Three rats of Cur-NPs-treated SAH group were for immunohistological staining at 24 h.
Results: Cur-NPs showed enhanced therapeutic effects than that of curcumin in improving neurological function, reducing brain water content and Evans blue dye extravasation after SAH. Mechanically, Cur-NPs attenuated BBB dysfunction after SAH by preventing the disruption of tight junction protein (ZO-1, Occludin and Claudin-5). Cur-NPs also up-regulated glutamate transporter-1 and attenuated glutamate concentration of cerebrospinal fluid following SAH. Moreover, inhibition of inflammatory response and microglia activation both contributed to Cur-NPs's protective effects. Additionally, Cur-NPs markedly suppressed SAH-mediated oxidative stress and eventually reversed SAH-induced cells apoptosis in rats.
Conclusions: Cur-NPs against SAH-induced BBB disruption may involve the prevention of glutamate-induced neurotoxicity, inflammatory responses, oxidative stress and cell apoptosis in the endovascular perforation rat SAH model.

PS06-010

Poster Viewing Session VI

Clinical and etiological profiles of adult patients with post stroke seizures in Bishkek

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Abstract
Background: Stroke is one of the most common cause of symptomatic epilepsy. Data for post stroke seizures and their natural history is lacking in developing countries due to absence of stroke registries. It is required special consideration in the elderly population due to comorbidity and altered pharmacokinetic. Moreover in some cases it is difficult to differentiate between acute provoke seizure caused by a stroke, acute neurological deficit caused by a seizure and seizure heralding stroke.
Purpose: The purpose of the study was to evaluate risk factors and clinical features of patients with post stroke seizures.
Methods: A consecutive 89 patients with post stroke seizures were evaluated, attending our neurology department between January and December 2015. The patients were studied for predictive factors and type of seizures following stroke.
Results: There were 39 F: 50 M. Age at seizure onset was between 11 and 79 years.
The most common identified etiological factors were aneurisms - 17,9%, AVM -15,4%, ischemic stroke −28,2%, hemorrhage stroke −38,5%.
The seizure types were generalized tonic clonic seizures - 29%, secondary generalized seizures - 45%, and simple partial seizures - 26%.
41% of seizures occur in the first 24 hours after stroke.
Risk factors for recurrent seizures were large cortical strokes, severe strokes, recurrent strokes and late seizures.
Conclusion: It is necessary to distinguish cause and consequence of seizure and stroke. EEG available 24 hours and MRI could be one of the emergency measures for that. Large cortical strokes, severe strokes, recurrent strokes and late seizures have a higher risk of recurrence of seizures, therefore could lead to post stroke epilepsy.

PS06-011

Poster Viewing Session VI

Direct carotid exposure for neuroendovascular approaches

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Abstract
Objective: The transfemoral approach is a common route for catheterization of the supra-aortic vessels in neuroendovascular therapy. However, in some cases, the patient's anatomy prevents transfemoral catheterization or distal access to the carotid s.In such cases, direct carotid

exposure (DCE) for neuroendovascular approaches may be used to treat cerebrovascular diseases.
Methods: We present 11 cases in which we were unable to perform the distal approach and DCE was the preferred neuroendovascular treatment procedure.
Results: DCE was performed on 11 patients with cerebral aneurysm (n = 8), carotid cavernous fistula (CCF) (n = 1), malignant brain tumor (n = 1), and carotid angioplasty and stenting (n = 1). Ten patients were female; one was male. Ages ranged from 63 to 87 years (mean: 71.36 years). Coil embolization was performed on patients with cerebral aneurysm and CCF. The patient with a malignant brain tumor underwent polyvinyl alcohol particle embolization. The only complication was a carotid artery dissection that occurred in one patient during stenting.
Conclusion: DCE for neuroendovascular approaches can be used as an alternative for patients with tortuous vasculature access in the femoral route. In such patients, a combination of neuroendovascular treatment and surgery in a hybrid operating room with angiography is preferred.

PS06-012

Poster Viewing Session VI

Xenon as a therapy in experimental intracerebral hemorrhage

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Abstract
Objectives: Preliminary studies have found xenon effective in experimental ICH.1 A full complement of STAIR studies is in progress to determine clinical potential, the first 5 of which are reported here.
Methods: Male C57Bl/6 J mice were subjected to collagenase-induced ICH and then:
Exp 1) Randomly received 0, 15, 30 or 45% xenon for 20 h beginning at 2 h post-injection;
Exp 2) 30% xenon for 0, 4, 8 or 20 h beginning at 2 h;
Exp 3) 30% xenon for 20 h beginning at 2, 4, 6, or 12 h;
Exp 4) comparison of efficacy in male and female mice; and
Exp 5) efficacy in the mouse autologous blood injection model. In all experiments, body weight and rotarod examined pre-injury, 1, 3, 7 and 28-days post-injury.
Neurological function2 and brain histology were examined (blinded) at 28-days post-injury.
Results:
Exp 1) All xenon doses improved 28-day outcome, with 30% most efficacious;
Exp 2) A minimum of 8 hours xenon exposure post-ICH was required for efficacy;
Exp 3) Xenon efficacy decreased with time to onset of treatment, 2 hours post-ICH was most effective;
Exp 4) Xenon produced similar levels of efficacy in both male and female mice;
Exp 5) Xenon improved autologous blood injection outcome, with the largest effect seen when treatment began 2 hours post-injection.
Conclusions: Xenon offer sustained improvement from ICH over 28 days in two different models. 30% xenon was optimal and treatment should be continued for at least 8 hours. Xenon was maximally efficacious when begun 2 hours post-ICH onset. Xenon was effective in males and females. We are now assessing the effect of xenon in aged rats and SHR rats before advancing to human trials.

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PS06-013

Poster Viewing Session VI

Prevalence of ischemic stroke in relation to season and its gender features in the northern part of Fergana valley of Uzbekistan

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Abstract
Purpose: To study season distribution of the ischemic stroke taking into account its type, gender and lethal outcomes on the territory of the northern part of Fergana valley in Uzbekistan.

Material and methods: There has been performed analysis of 616 medical histories of patients hospitalized to the branch of Republican Scientific Center For Emergence Medical Aid of Namangan Province of Uzbekistan of the age from 32 to 82 (average age 60,0 ± 4,7). Of them men were 271 and women −345.
Results of research: Depending on the month maximal distribution of Ischemic stroke(IS) was noted in April among men, and in January among women (10,9 and 9,9%, respectively). Minimal distribution of IS was noted in men in January and among women in November (6,3 and 7,0%, respectively). Depending on season prevalence of the IS had special features and regularities. Among men maximal number of cases of IS was found in summer, and minimal - in winter (28 and 21,7%, respectively). In women IS distributed evenly in all seasons.
With regard to type of stroke and gender differences there were also determined some regularities in distribution of the lethal outcomes in different months. Among men in (IS) the maximal number of lethal outcomes was noted in spring, and minimal - in summer (32,2 and 16,9%, respectively). In women with IS maximal cases of lethal outcomes was registered in autumn, and minimal - in summer (30,1 and 20,5%, respectively).
Conclusion: Thus, IS in men was registered frequently in summer, whereas among women its distribution was even during whole year. In men maximal number of lethal outcomes due to IS was determined in spring At the same time among women the maximal number of dead patients was noted in autumn.

PS06-014

Poster Viewing Session VI

Stroke patterns in rural South India - Five years experience in a rural stroke centre

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Abstract
Objectives: Our Aim was to study the incidence of stroke among general neurologic patient population, to identify the stroke subtypes, to identify the prevalent risk factors.
Methods: In this retrospective study, data were obtained from our hospital registry and STROKE REGISTRY from 01/04/2011 to 31/03/2016. All patients who presented to our Emergency room with symptoms of stroke were included in the study.
Results: The number of patients who got admitted with ischemic strokes were 1728 (67% of total stroke). Male were 1164(67%) and Female 564 (32.63%), Age range was (1 ½ yrs- 91 yrs).Risk factors included Diabetes mellitus in 414 (24%), Systemic Hypertension in 324(19%), Dyslipidemia in 327(19%), Rheumatic Heart Disease in 72 (4%). Out of 1164 Male patients, 696 (40%) patients were tobacco and alcohol users. Out of the 1728 total Ischemic stroke patients , 948 (54%) patients had large artery stroke in Anterior Circulation, 525 patients (30%) had posterior circulation strokes & 255 patients (14%) had lacunar strokes. 225 patients (13%) had come within the time window and were eligible for thrombolysis, & 126 of them (7.5%) underwent successful IV thrombolysis.Intra cerebral Hemorrhage accounted for 483 patients (18%), with a age range of (14 yrs–80 yrs). Hypertension was present in 300 (62%) and Diabetes mellitus in 75 (15%). Out of the 483 ICH, 363 (75%) were pure parenchymal ICH, ICH with IVH were seen in 87 (18%). Pure IVH in 33 (6%) patients.
Cortical venous sinus thrombosis accounted for 228 (9%) of the total stroke population. Age range of (6 yrs–73 yrs).
Conclusions: This study shows that the incidence of strokes and stroke sub types not very different from other parts of India. Cortical venous thrombosis has a higher incidence. IV thrombolysis can be achieved in rural India with comparable rates with proper infrastructure and training.

PS06-015

Poster Viewing Session VI

MiR-210 boosts the protective effects of NPC-EXs on neurons and astrocytes against hypoxia/reoxygenation-induced injury

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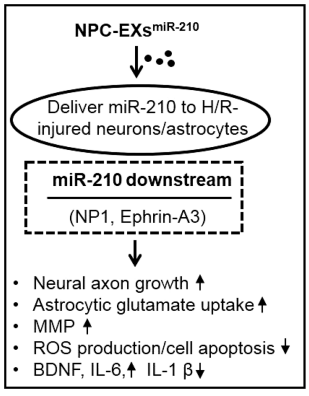
Abstract
Transplantation of neural progenitor cells (NPCs) has therapeutic effects on ischemic stroke. Increasing evidence suggest that extracellular exosomes (EXs) mediate the beneficial effects of stem cells by delivering microRNAs (miRs) to target cells. It has been shown that miR-210 offers anti-apoptotic effect on neurons and its circulating level positively correlates with outcome of stroke patients. Here, we investigated whether miR-210 enriched NPC-EXs (NPC-EXs$^{miR-210}$) have better protective effects on neurons/astrocytes subjected to hypoxia/reoxygenation (H/R) injury. NPC-EXs$^{miR-210}$ or NPC-EXs$^{miR-con}$ were collected from NPCs transfected with miR-210 mimics or miR-control. Neurons/astrocytes were subjected to hypoxia (1% 0_2) for 6 hrs, followed by reoxygenation with application of vehicle, NPC-EXs$^{miR-con}$ or NPC-EXs$^{miR-210}$ (50 ug/ml) in culture medium. After 24 hrs, neuronal axon length and glutamate uptake ability of astrocytes were evaluated. Cell apoptosis (TUNEL assay), mitochondria membrane potential (MMP; JC-1 staining), and ROS production (DHE staining) were analyzed. The expressions of miR-210 downstream molecules (neuronal pentraxin1/NP1, ephrin-A3) and apoptotic genes (cyt c, caspase- 3) in cells, and concentrations of cytokines (BDNF, IL-1β, IL-6) in culture media were determined by western blot or ELISA. Results showed: 1) The level of miR-210 in NPC-EXs$^{miR-210}$ was 10-fold of that in NPC- EXs$^{miR-con}$, and NPC-EXs$^{miR-210}$ upregulated miR-210 level in H/R-injured neurons/astrocytes; 2) Compared to vehicle, NPC-EXs$^{miR-con}$ decreased H/R-induced neural and astrocytic apoptosis and dysfunctions (axon outgrowth and glutamate uptake ability), which were associated with significant downregulation of NP1/ephrin-A3, increase of MMP, BDNF and IL-6 release, decrease of ROS production, cyt c, cleaved caspase-3, IL-1β; 3) NPC-EXs$^{miR-210}$ were more effective than NPC-EXs$^{miR-con}$ on all of these effects, except for changes in IL-6 and IL-1β. Taken together, our data demonstrate that miR-210 enrichment can enhance the protective effects of NPC-EXs on neurons/astrocytes from H/R injury through targeting the NP1/ephrin-A3 pathways (Fig 1).



[Fig 1]

PS06-016

Poster Viewing Session VI

MicroRNA miR-21 is neuroprotective against focal cerebral ischemia

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Abstract
Objectives: The microRNA miR-21 has been seen to promote cellular survival in a variety of paradigms, including traumatic brain injury, cardiac ischemia, and in vitro models of stroke. This is attributed to its ability to prevent the translation of pro-apoptotic targets such as programmed cell death factor 4. In the present study, we evaluated the therapeutic potential of miR-21 administration in a murine model of ischemic stroke.
Methods: Degradation-resistant miR-21 mimic or scramble control in a PEGylated liposome vehicle was injected intracerebrally into adult male C57BL/6J mice 2 h prior to a 1 h transient middle cerebral artery occlusion (MCAO). At 2 days of reperfusion, infarct volumes were measured using TTC-stained serial brain sections. Total RNA isolated from cortical penumbral tissue was used for real-time PCR analysis of miR-21 and an array of its downstream targets.
Results: The miR-21 mimic treated group showed a significantly higher levels of miR-21 (by 59.7 fold; $p < 0.05$), smaller infarct volume (by ~30%, $p < 0.05$; $n = 9$/group), and decreased body weight loss (by ~32%; $p < 0.05$) compared to control mimic group. The miR-21 mimic treatment also resulted in decreased expression of several previously validated miR-21 targets that promote apoptosis.
Conclusions: MiR-21 is a potent promoter of cell survival after stroke. The degradation-resistant miR-21 mimic persists in the brain and prevents the expression of apoptotic proteins, potentially stabilizing the penumbra. MiR-21 mimic can be a novel neuroprotective therapy for stroke.

PS06-017

Poster Viewing Session VI

Silencing of deSUMOylating isopeptidase 2 increases neuronal vulnerability after ischemic-like stress

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Abstract

Objectives: Conjugation of small ubiquitin-like modifier (SUMO) to target proteins is an important post-translational modification capable of altering a wide variety of cellular functions and, consequently, cell fate. Modulation of the SUMOylation of particular proteins has often been implicated in the response to stress conditions, including ischemia. A new class of SUMO proteases, deSUMOylating isopeptidase (DESI), has recently been identified. In the present work, we are investigating the functions and relevance of DESI2 in neurons, its target proteins of deSUMOylation and its role in the endogenous neuroprotection against ischemia.

Methods: Endogenous expression of DESI was analyzed in the mouse brain and cultured cortical neurons by immunostainings. Silencing of DESI variant 2 expression in primary cultures of mouse cortical neurons was achieved by neuronal-specific shRNA interference delivered by lentiviral particles. The cultures were then exposed to hypoxic conditions in a damaging oxygen-glucose deprivation (OGD) model for 120 minutes. The survival of neurons was assessed over time by microscopic observation of the expression of reporter gene EGFP and propidium iodide inclusion.

Results: DESI2 is endogenously expressed throughout the mouse brain, remarkably in the cortex and hippocampus. In cultured neurons, it is present both in the nucleus and cytoplasm and co-localizes with SUMO. Concentration-dependent knockdown of DESI2 by shRNA interference was confirmed on the protein level by Western Blot. DESI2-depleted neurons showed significantly lower survival rates after OGD compared to the pre-OGD condition than neurons expressing a non-targeting EGFP control shRNA.

Conclusions: DESI2 seems to be necessary for the full endogenous protection capacity of neurons against ischemic-like stress. We now aim at rescuing neuronal survival by enhancing DESI2 expression. Upcoming proteomics analysis will reveal the target proteins of deSUMOylation by DESI2 and indicate the possible cellular mechanisms involved.

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PS06-018

Poster Viewing Session VI

Microglial-conditioned media treated with geniposide and ginsenoside Rg1 on ischemic neurons

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Abstract

The synergistic mechanism underlying the effects of multi-component combined drug use for complex diseases remains to be fully elucidated. Microglial activation following ischemia can either affect neural survival or cause neuronal injury. The aim of the present study was to determine the synergistic effect of geniposide and ginsenoside Rg1, based on microglial-neuronal communication. N2a neuronal cells were divided into the following seven groups: Control group; normal cultured microglial cells in conditioned medium (N-MG-CM) group; oxygen-glucose deprivation (OGD) model group; OGD-injured MG-CM (I-MG-CM) group; geniposide-treated MG-CM (G-MG-CM) group; ginsenoside Rg1-treated MG-CM (R-MG-CM) group; and combination-treated MG-CM (C-MG-CM) group. A series of assays were used to detect the effects of the different MG-CM on neurons in terms of: (i) cell viability, determined using a Cell Counting Kit-8; (ii) lactate dehydrogenase (LDH) leakage rate; (iii) expression of NMDAR1 and activated caspase-3, detected using western blotting; (iv) mitochondrial transmembrane potential, determined by JC-1; and (v) mitochondrial ultrastructural features, determined using electron microscopy. The

experimental results demonstrated that MG-CM including the integrated use of geniposide and ginsenoside Rg1 significantly protected neuronal cell viability and inhibited LDH leakage, suppressed the expression of N-methyl-D-aspartate receptor subunit 1 and activated caspase-3, increased the mitochondrial transmembrane potential and improved the mitochondrial ultrastructure. MG-CM from separately used geniposide or ginsenoside Rg1 demonstrated differential neuroprotection at different levels. These findings revealed that the synergistic drug combination of geniposide and ginsenoside Rg1 in the treatment of stroke is a feasible approach for use.

PS06-019

Poster Viewing Session VI

Investigating the effects of chronic stress on the neurovascular unit

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Abstract
Objectives: Chronic distress impairs stroke outcome and elevates stroke risk. We evaluated molecular mechanisms that may be involved in a dysfunctional neurovascular unit as a consequence of stress.
Methods: To investigate effects of distress *in vivo* we performed two different stress models in 129SV mice. The chronic stress model, which was carried out for 28 days, consisted of three different stressors: exposure to rat, restraint stress and tail suspension. In the acute stress model, mice were exposed only once to the rat. Experiments with hippocampal slices and primary cortical neurons were performed to evaluate effects of stress and glucocorticoid signaling *in vitro*.
Results: After 28 days of chronic stress, mice showed significantly higher adrenal weights but reduced corticosterone levels in comparison to the unstressed controls. After acute stress, the animals showed increased corticosterone concentrations in comparison to the control group. Stimulation with dexamethasone alone or in combination with OGD regulated the glucocorticoid receptor as well as Fkbp5 mRNA expression *in vitro*.
Conclusion: Our chronic stress model leads to a dysfunctional HPA axis. Dexamethasone treatment regulates the glucocorticoid receptor and Fkbp5 mRNA expression in hippocampal slices and cortical neurons which could be important for function of the neurovascular unit in the context of stroke.

PS06-020

Poster Viewing Session VI

Differential protein expression profiling of focal cerebral after human cerebral endothelial cell transplantation

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Abstract
Objective: Cerebral endothelial cells have unique biological features and are fascinating candidate cells for stroke therapy.
Materials and methods: In order to understand the molecular mechanisms of human cerebral endothelial cell (hCMEC/D3) transplantation in a rat stroke model, we performed proteomic analysis using 2-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Protein expression was confirmed by qRT-PCR and Western blot.
Results: Several protein spots were identified by gel electrophoresis in the sham, cerebral ischemia (CI), and CI with hCMEC/D3 treatment (CT) groups, and we identified 14 differentially expressed proteins in the CT group. Proteins involved in mitochondrial dysfunction (paraplegin matrix AAA peptidase subunit, SPG7), neuroinflammation (peroxiredoxin 6, PRDX6), and neuronal death (zinc finger protein 90, ZFP90) were markedly reduced in the CT group compared with the CI group. The expression of chloride intracellular channel 4 (CLIC4) proteins involved in post-ischemic vasculogenesis was significantly decreased in the CI group but comparable to sham in the CT group.
Conclusion: These results contribute to our understanding of the early phase processes that follow cerebral endothelial cell treatment in cerebral ischemia. Moreover, some

of the identified proteins may present promising new targets for stroke therapy.

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science & Technology Development" and National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. NRF-2016R1A2B4008316).

PS06-021

Poster Viewing Session VI

Elevating MicroRNA-122 in blood improves outcome via modulating Nos2 after middle cerebral artery occlusion in rats

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Abstract

Based upon our previous findings that microRNA-122 (miR-122) was decreased in peripheral blood of both humans and rats after ischemic stroke, we hypothesized that elevating miR-122 in blood might improve outcomes after ischemic stroke.

Using the *in vivo* polyethylene glycol 2000 (PEG)-liposome based miRNA transfection system and the rat middle cerebral artery occlusion (MCAO) model, we recently demonstrated that intravenous (i.v.) miR-122 mimic, given immediately after MCAO, elevated miR-122 in peripheral blood, prevented neurological impairments, and reduced brain infarction volume by 90% after MCAO in rats. Moreover, the results showed that miR-122 mimic, given 6 hr after MCAO, attenuates neurological impairments, and reduced brain infarction volume by 54% after MCAO in rats.

Using Taqman PCR based assays, we demonstrate fourteen direct miR-122 target genes (e.g. Nos2, Vcam1, Clic4, Ucp2, Dlg2, and others) decrease in blood leukocytes following miR-122 mimic treatment after MCAO in rats. Focusing on ONE miR-122 target gene (Nos2), we demonstrate that miR-122 mimic decreases Nos2 expression in brain microvascular endothelial cells (BMVECs) after MCAO in rats.

These results show that Nos2 is decreased in leukocytes and BMVECs following miR-122 mimic treatment after MCAO, which likely contributes to therapeutic effects of miR-122 mimic on ischemic stroke.

Acknowledgements: This study was supported by NIH grants R01NS089901 (DZL), R01NS054652 (FRS) and AHA Fellow-to-Faculty Transition Award (GCJ). There were no conflicts of interest.

PS06-022

Poster Viewing Session VI

The EphB/ephrinB receptor -ligand system promotes the inflammatory response upon ischaemia

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Abstract

Ischaemic strokes are within the most common causes for disability in the industrialized world. The EphB/ephrinB system system causes bi-directional signal transmission and is known for the activation of glial cells, gliosis, and the transmigration of activated monocytes. Nevertheless, little is known about the mechanisms and involvement of this system in the inflammatory response in the acute and early subacute phase upon an infarction.

RNA-microarray analysis using EphB2 deficient and wild type mice subjected to transient middle cerebral artery occlusion (tMCAo) showed a stronger inflammatory response in EphB2 holding animals. Nevertheless, no differences in the transmigration of neutrophils were observed between the genotypes. Further, microglia and astrocytes did not show differences in the abundance, migration or proliferation between the genotypes. Additionally, a bead assay with primary cultured murine microglia showed no influence of the EphB/ephrinB system on the phagocytic activity. However, primary microglia and astrocytes do react to hypoxic conditions in combination with glucose deprivation by increasing the expression of the membrane-bound ligand ephrin-B2. When, in turn, stimulated with EphB2, astrocytes increase their expression of pro-inflammatory cytokines and thus become activated. In contrast, high amounts of cytokines in the medium decrease the ephrinB expression in astrocytes suggesting a self-regulating mechanism. These results

indicate a pro-inflammatory role of the EphB/ephrinB system upon stroke. Indeed, *EphB2*$^{-/-}$ mice show significantly smaller infarction and oedema upon tMCAo. The functional outcome of these mice was consistent with this difference.

In summary, the EphB/ephrinB system is involved in the reaction to ischaemia in the brain and in immunoactive glia cells in particular. Astrocytes do react to EphB2 in a pro-inflammatory manner. Moreover, microglia and astrocytes increase their ephrin-B-ligand expression upon hypoxia, which potentially makes them more responsive to the EphB2 receptor. The exact cellular mechanism by which ephrin-B2 evokes possible detrimental effects remains to be identified.

PS06-023

Poster Viewing Session VI

Characterization of glial cells during the chronic stage of ischemic stroke in rats

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Abstract

Objectives: It is important to emphasize that many new lines of evidence point towards numerous essential beneficial functions of reactive astrogliosis and scar formation, particularly as regards neural protection and repair, and regulation of neuroinflammation. So, we explored the characteristics of glial cells in the peri-infarct area of chronic ischemic stroke Wistar rats.

Methods: Male Wistar rats were randomly divided into a normal group, a sham-operation group and a model group. Ischemic stroke models were established in the model group by phtothrobotic ischemia of motor and scensosry motor cortex area. The pathological changes of glial cells in rat's brain tissue were dynamically observed at 1 day, 1, 2, 4, 6, 8, 10, and 12 weeks after cerebral ischemia by using histopathologic staining and immunohistochemical staining.

Results: Using immunohistochemistry for GFAP in stroke animals, we found that the activation of astrocytes were increased at 1 day, 1 week, and 2 weeks in the ipsilateral cortex after stroke and the appearance of the astroglial scar in peri-infarct regions at 4 weeks was occured and continued at 6, 8, 12 weeks. Especially astrogliosis and activation of astrocytes were increased at 1 week after stroke in the contralateral cortex. We also found that elongated astroglia in the peri-infarct area expressed the progenitor marker Sox2 and the radial glial marker Blbp (brain lipid binding protein), and STAT3 at 1 week, and 2 weeks after stroke. The increased levels of Sox2, Blbp, and STAT3 expression were downregulated at 4, 6, 8, and 12 weeks after stroke. M2 levels exerted anti-inflammatory effects on microglia were increased at 1, 2, 4, 6, 8, 12 weeks after stroke.

Conclusion: Further studies are needed to elucidate the functions of reactive astrogliosis, scar formation, and microglia activation after stroke.

PS06-024

Poster Viewing Session VI

Effect of RGS5 on vasculature remodeling after experimental stroke

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Abstract

Objectives: Stroke is the third leading cause of disease mortality worldwide and currently, there is no effective pharmacological treatment available for stroke, beyond the acute thrombolysis. Much of the underlying molecular mechanism of repair after ischemic stroke is not known. Brain pericytes, perivascular cells in the brain, have multiple roles in microcirculation, angiogenesis and maintenance of the blood-brain-barrier. Therefore, pericytes may be a key player in brain ischemia. We have shown that regulator of G-protein signaling 5 (RGS5)-expressing pericytes are activated after experimental stroke (1). RGS5 has been implicated to be involved in fetal vascular maturation (2) and in angiogenesis in tumor vessel formation (3). However, the contribution of RGS5 to rebuild the vasculature after stroke has not been investigated so far.

Method: To study the role of RGS5 in angiogenesis after stroke, we use a *Rgs5* knockout mouse strain that expresses GFP under the *Rgs5* promoter (4). The mice are exposed to permanent middle cerebral arterial occlusion (pMCAO) and sacrificed 7 days after stroke.
Results: We show that *Rgs5* knockout mice ($Rgs5^{GFP/GFP}$) have a higher pericyte coverage of the vasculature than *Rgs5* heterozygous mice ($Rgs5^{GFP/+}$) 7 days after experimental stroke. Preliminary results suggest that the deletion of RGS5 is involved in vascular repair.
Conclusions: RGS5 may be a new target for vascular modulation after stroke.

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PS06-025

Poster Viewing Session VI

Longitudinal metabolic evolution of rat cortex

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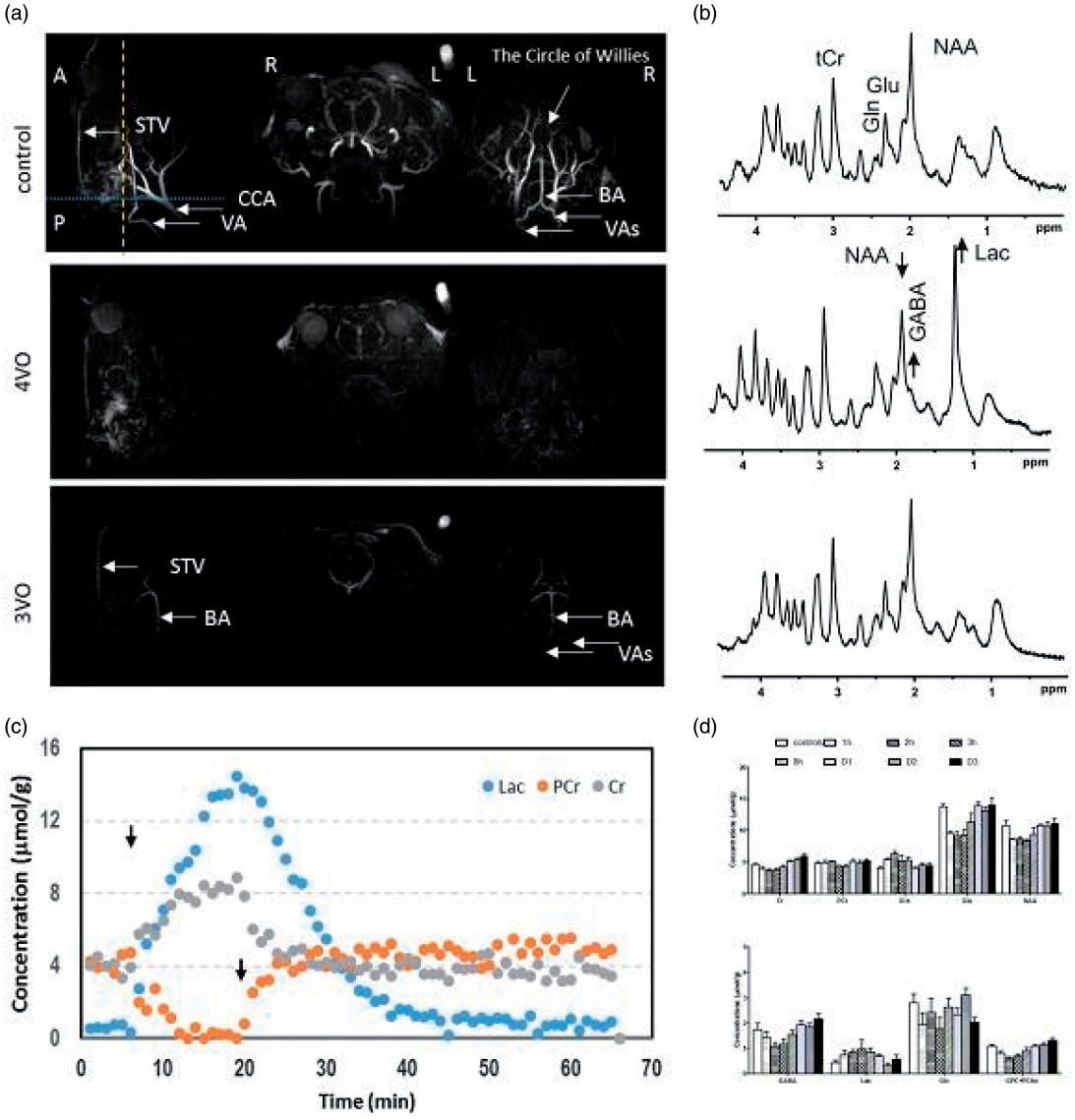
Abstract
The ability of 1H MRS to identify stroke damage, predict stroke outcome (neuronal loss or recovery) and estimate ischemic onset times based on metabolite concentrations has been shown. In this study, we aimed to evaluate whether localized 1H MRS at ultra-short time in combination with another MR method, namely MR angiography (vasculature images), could be applied for diagnosis purpose on rat upon global ischemia (4-VO) in a 14.1T magnet. The second aim of this study was to assess longitudinal metabolic evolution of cortex upon a short period of transient global ischemia.
We applied one-stage anterior approach to achieve 4VO in adult male Wistar rats (300–350 g). The MIP images illustrated the typical vascular structure of rat head, i.e. CCAs, VAs, BA and the circle of willies etc (Figure 1a). After 4VO, the MIP images showed that nearly all signals originated from major arteries, were significantly diminished (Figure 1a). 1H MRS showed strong changes upon a compelete 4VO (Figure 1b). We further studied animals before, during and after a complete 15-min 4VO. Once the vertebral arteries were occluded completely, 1H MRS of cortex, a 1-min temporal resolution of metabolite evolution was obtained. Once 4VO was achieved remotely, phosphocreatine (PCr) dropped, creatine (Cr) and lactate (Lac) elevated (Figure 1c). When reperfusion was restored by releasing two occluders, PCr recovered while Cr dropped. With the restoration of perfusion, lactate decreased gradually. Further evolution of these metabolites was also observed in animals hours (1 h, 2 h, 3 h and 8 h) and days (D1, D2 and D3) after such brief ischemia (Figure 1d). A transient elevation of glutamine (Gln) was observed after the restoration of perfusion, consistent with excitoxicity, which was reported in mice after transient focal ischemia.
We conclude that 1H MRS assessment of cortical metabolite changes during and immediately after cerebral ischemia is feasible.



PS06-026

Poster Viewing Session VI

B cell-mediated stroke recovery in female mice

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Abstract

Background: We recently found that B cell depletion prior to stroke in adult male mice impedes motor recovery, stoke-induced neurogenesis, and increases hippocampal-dependent cognitive deficits. However, the role of B cells during long-term stroke recovery in adult female mice is not known. Therefore, we hypothesized that B cells mediate functional recovery in female mice following stroke.

Methods: Motor activity of female transgenic (12–16 week old) mice expressing human CD20 (hCD20+) on B cells, or CD20-null littermates (WT) was assessed on rotarod over a 2-week training prior to stroke. Rituximab was administered (hCD20+ depleting drug) daily 3 days prior to 60-minute transient middle cerebral artery occlusion and weekly thereafter. Motor function was assessed at 2, 7, and 14 days post-stroke. Brain

tissue was collected for histology (infarct volume, neurogenesis, and angiogenesis), spleens for flow cytometry, and serum for estrogen quantification. Pre- and post-stroke motor function was analyzed by two-way ANOVA, Bonferroni post-hoc (Graphpad Prism). B cell depletion was assessed by flow cytometry (FlowJo v9) and analyzed by t-test, Mann-Whitney. Significance was $p < 0.05$.

Results: No effect of genotype was observed in motor function during the 2-week training prior to stroke. By two-way ANOVA, only hCD20+ mice exhibited long-term deficits at day 14 post-stroke. On day 14, motor deficit of hCD20+ mice was worse compared to WT mice ($p < 0.05$). B cell depletion reduced the population percentage ($p < 0.01$) and absolute cell count ($p < 0.0001$) of splenic B cells in hCD20+ mice compared to WT. While percentages of other splenic innate and adaptive immune cells increased ($p < 0.01$), B cell depletion did not affect absolute cell counts of these populations between genotype.

Conclusions: Coinciding with previous stroke data in males, our results show that B cells play an important role in modulating post-stroke recovery in female mice independent of an estrous cycle.

PS06-027

Poster Viewing Session VI

The double edged sword of immunostimulation after stroke

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Abstract

Objectives: Pulmonary infection is a highly relevant complication and leading cause of death in patients after acute CNS injury, including ischemic stroke. The high incidence of pneumonia in these patients is likely to be the result of a temporary stroke-induced immunodepression. Recent clinical trials demonstrated that preventive antibiotic therapy fails to reduce the incidence of pneumonia and to improve outcome. Hence, new therapeutic avenues to improve pulmonary host defences may be required to prevent development of bacterial infections and improve outcome. Pre-activation of the pulmonary immunity by local or systemic administration of immunostimulatory cytokines or Toll-like receptor ligands is one such approach. Due to the compromised blood-brain barrier after stroke a stimulation of the immune system may also increase inflammatory responses in the ischemic brain and thereby worsen the neurological outcome.

Methods: In a mouse model of focal cerebral ischemia we analysed the efficacy and safety of different local immunostimulatory treatments including application of GM-CSF, IFNg and the TLR2 ligand MALP-2 to reverse stroke-induced susceptibility to pulmonary bacterial infections. To determine whether local treatment can be safely applied without adverse neurological side-effects we investigated brain lesion development and neurological long-term outcome by MRI and assessing focal neurological malfunction and motor function by gait analysis.

Results: Whereas subcutaneous GM-CSF application did not reduce the incidence of pneumonia it prevented translocation of bacteria. Additionally, subcutaneous GM-CSF treatment reduced infarct size and restored leucocyte numbers in peripheral blood. Assessment of neurological long-term outcome to determine whether subcutaneous GM-CSF therapy can be safely applied, showed significantly improved movement and sensorimotor functions. GM-CSF treated mice showed a symmetric corner turning and regained comparable motor function than before stroke.

Conclusions: In a model of aspiration-induced pneumonia local GM-CSF therapy failed to reduce the incidence of pneumonia whereas subcutaneous GM-CSF therapy improved movement and sensorimotor functions.

PS06-028

Poster Viewing Session VI

Periodontitis modulates peripheral inflammatory cell function and contributes to worse outcome after ischaemic stroke

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Abstract

Objectives: Periodontitis (PD) is an extremely common chronic inflammatory disease that affects the supporting structures of the teeth. PD is also a risk factor for a multitude of systemic disorders, including cardiovascular disease and stroke. While many epidemiological studies

suggest that PD increases the risk of stroke, it is not currently understood if PD contributes to worse outcome or impaired recovery after stroke. Given the widespread prevalence of PD, and the considerable morbidity and mortality associated with stroke, it is important to determine the extent that PD influences stroke-induced pathology.
Methods: An acute ligature-induced mouse model of PD was first characterised, assessing local and systemic inflammation. Employing this model, the influence of PD on stroke was subsequently examined, to test the hypothesis that PD worsens outcome after stroke.
Results: Ligature-induced PD caused alveolar bone loss and a significant increase in the inflammatory infiltrate in the local sub-mandibular lymph nodes, but did not elicit a robust inflammatory response at peripheral sites such as the lung, spleen, or blood. In the bone marrow, however, PD modulated neutrophil and monocyte frequency and also primed inflammatory monocytes to produce TNFα. In the context of stroke, our data suggest that PD exacerbates ischaemic brain damage at 48 hours post-stroke. At present a mechanism for this exacerbation is not known as there were no changes in peripheral immune cell function or migration. Preliminary data suggest, however, that PD may exert an effect on the vasculature and this could be a possible contributor to the risk of stroke and damage after stroke.
Conclusions: Collectively, these findings suggest that PD negatively affects outcome after stroke and may contribute to poorer long-term recovery following ischemic brain injury.

PS06-029

Poster Viewing Session VI

The dynamics of energy metabolism in brain after ischemia/reperfusion injury: an animal model study using high-Gz acceleration

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Abstract
Objectives: The dynamics of high energy phosphates (ATP, ADP and AMP) of mice brain was observed after cerebral ischemia/reperfusion (IR) injury induced by exposure to high +Gz acceleration using a centrifugal acceleration device for small animals.
Methods: Twenty mice (C57BL/6NCrSlc, BW 25.4 ± 1.4 g) were exposed to +8 Gz acceleration (from rostral to caudal) for 30 s to induce IR injury. Cerebral blood flow (CBF), cerebral tissue partial pressure of oxygen (CP_{O2}) and heart rate (HR) were monitored. The brain was *in-situ* frozen immediately (IIR, $n = 5$), 5 min (5IR, $n = 6$) and 10 min (10IR, $n = 4$) after IR. Samples frozen before IR served as control (Con). Serial coronal sections (10-μm) were made with a cryomicrotome and thaw-mounted on ITO-coated glass slides. Imaging mass spectrometric analysis was performed by a MALDI TOF/MS (AXIMA; Shimadzu Co, Kyoto, Japan) with 9-aminoacridine as the matrix. Relative levels of high energy phosphates and energy charge (EC) were calculated as indices of energy metabolism of the brain.
Results and Conclusion: High +Gz acceleration decreased CBF, CP_{O2} and HR to 17%, 43% and 46% of the baseline, respectively. CBF increased significantly soon after the device stopped while HR recovered to the baseline level. On the contrary, the recovery of CP_{O2} was delayed by up to 3 min. In IIR, AMP was significantly decreased ($p = 0.036$) while ATP had already recovered to the baseline level and EC showed insignificant increase ($p = 0.076$). ATP, ADP, AMP and EC were all in the baseline level in 5IR and 10IR. These findings might indicate possible rapid recovery of energy metabolism of brain tissue immediately after IR injury probably due to the rapid recovery of CBF, regardless of the delayed recovery of CP_{O2}.

PS06-030

Poster Viewing Session VI

Efficacy of Alteplase® in a mouse model of acute ischemic stroke: a retrospective pooled analysis

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Abstract

The debate over the fact that experimental drugs proposed for the treatment of stroke fail in the translation to the clinical situation, has attracted considerable attention in the literature. In this context, we present a retrospective pooled analysis of a large dataset from pre-clinical studies, in order to examine the effects of early versus late administration of intravenous recombinant tissue type plasminogen activator (rt-PA).

We collected data from 26 individual studies from 9 international centers (13 researchers, 716 animals) that compared rt-PA to controls, in a unique mouse model of thromboembolic stroke induced by an *in situ* injection of thrombin into the middle cerebral artery. Studies were classified into early (< 3 h) versus late (≥ 3 h) drug administration. Final infarct volumes, assessed by histology or MRI, were compared in each study and the absolute differences were pooled in a random-effect meta-analysis. The influence of time of administration was tested.

When compared to saline controls, early rt-PA administration was associated with a significant benefit (absolute difference $= -6.63$ mm3; 95% CI, -9.08 to -4.17; $I^2 = 76\%$) whereas late rt-PA treatment showed a deleterious effect (+5.06 mm3; 95% CI, +2.78 to +7.34; $I^2 = 42\%$, $P_{int} < 0.00001$). Results remained unchanged following subgroup analyses.

Our results provide the basis needed for the design of future pre-clinical studies on recanalization therapies using this model of thromboembolic stroke in mice. The power analysis reveals that a multi-center trial would require 123 animals per group instead of 40 for a single center trial.

PS06-031

Poster Viewing Session VI

LabCIRS - a lightweight critical incident reporting system for academic research departments

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Abstract

Recent meta analyses show that insufficient reproducibility of preclinical studies leads to serious drawbacks in translation from experimental research to clinical treatment. Based on the experience from clinical studies, multiple guidelines for the improvement of the reseach quality in academic biomedical research laboratories emerged. Among many others measures, implementation of error management systems, within or outside the context of structured quality management systems was suggested.

Errors, mishaps, and mistakes of variable severity frequently occur in biomedical research laboratories. Although errors can negatively impact data integrity, experimental outcomes, animal welfare, personnel safety, or viability of expensive reagents or machinery, they are reported only sporadically or erratically, or might even be covered up for fear of negative consequences.

To improve our error culture we decided to implement an anonymous critical incident reporting system (CIRS), which has become a standard in clinical medicine but has to our knowledge never been implemented in the context of academic basic research.

We developed, tested and implemented LabCIRS, a simple, open-source software tool written in the Python programming language as a user friendly, simple on-line critical incident reporting system for research groups, laboratories or institutions. LabCIRS is accepted by all members of the department, has led to the emergence of a mature error culture, and has made the laboratory a safer and more communicative environment. Initial concerns that implementation of such a measure may lead to a 'surveillance culture' which would stifle scientific creativity turned out to be unfounded.

PS06-032

Poster Viewing Session VI

Modulation of the peri-infarct neurovascular function by β-Hydroxybutyrate

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Abstract

Due to the oxygen dependence of the glycolytic pathway [1], production of ATP in ischemic tissue is accompanied by generation of Reactive Oxygen Species (ROS). The aim of this work was to test the efficacy of providing a ketone body, an alternative metabolic substrate, in the acute post-ischemic phase so as to reduce ROS production and improve neurovascular recovery.

To examine the susceptibility of the neurogliovascular unit to metabolic modulation in the acute stage of focal ischemic stroke, we delivered a ketone body (β-Hydroxybutyrate, BHB; 100 mg/kg i.p.) one hour after ischemic insult induced by direct cortical microinjection of endothelin-1 in sensorimotor cortex of adult rats (800 pico moles, as in our previous work [2]). We contrasted functional vascular responses to hypercapnia imaged on continuous arterial spin labeling (CASL) MRI and resting state field potentials in BHB- *vs.* vehicle-treated cohort 48 hours after stroke induction.

In line with our previous study [2], transient hypercapnia in the vehicle treated cohort elicited exaggerated vascular functional responses in the peri-ischemic tissue (1.8 ± 0.5 times larger in lesioned *vs.* un-lesioned hemisphere). In contrast, BHB treated cohort shows reduced lateralization of the CBF increase. After CASL experiments, resting state local field potentials showed decreased theta-band power in the peri-lesional tissue (0.75 ± 0.22 mV/mV*Hz-1 lesioned/un-lesioned hemisphere), which was partially rescued (0.91 ± 0.02 mV/mV*Hz-1 lesioned/un-lesioned hemisphere) in the BHB-treated cohort.

Our data suggests that BHB administration may be beneficial for the neurovascular unit recovery. As opposed to other neurocentric approaches, our project shows the efficacy of a pleiotropic treatment that may benefit multiple cells in the neurovascular unit.

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PS06-033

Poster Viewing Session VI

Hematoxylin-eosin stained pathology of hyperacute focal cerebral ischemia at 1 hour after middle cerebral artery occlusion in rats: correlation with diffusion MRI

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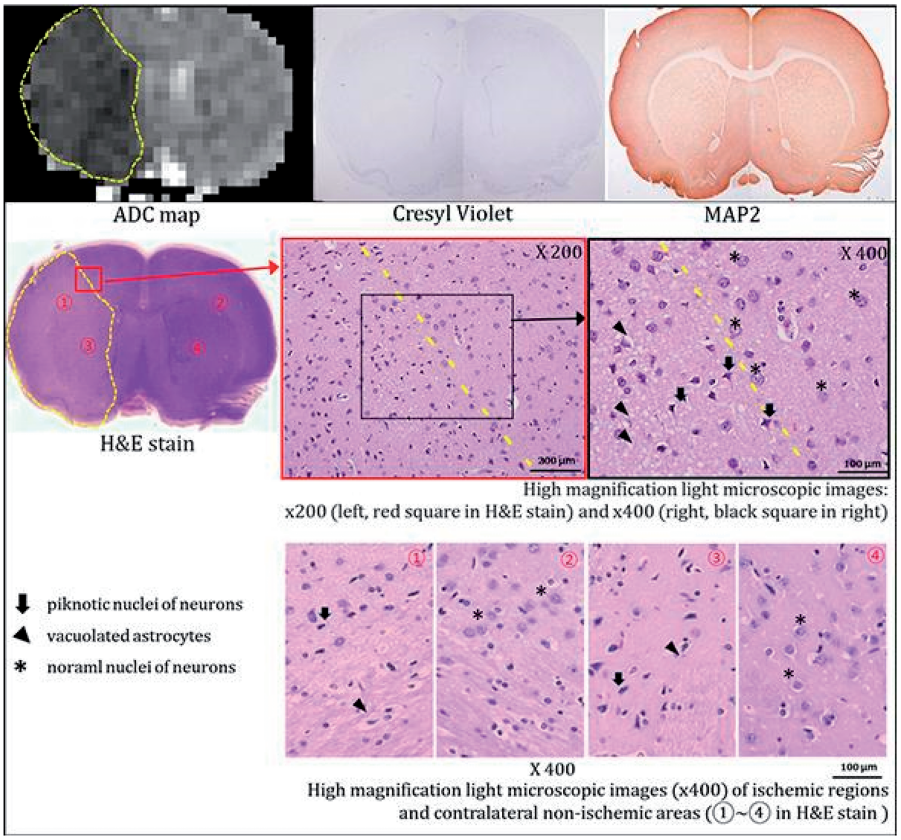
Abstract

Purpose: To correlate gross and microscopic pathology of hyperacute focal cerebral ischemia at 1hour after permanent middle cerebral artery occlusion (MCAO) with diffusion MRI (DWI) in rats.

Materials & Methods: Thirty-three Sprague-Dawley rats were examined with prospective 3 Tesla DWI (axial, every 10 minutes to 55 minutes, 5 times) and dedicated animal coils immediately after right MCAO. We selected the animals showing sustained diffusion restriction in right middle cerebral artery (MCA) territories on apparent diffusion coefficient (ADC) map, and each was followed by single coronal DWI for the comparison with gross pathology. Brain sections (coronal, 2 mm in thickness) were treated

with hematoxylin-eosin (H&E), Cresyl violet, microtubule-associated protein 2 (MAP2), terminal deoxynucleotidyl-transferase- mediated dATP nick end labeling (TUNEL) staining methods for gross and microscopic evaluation.
Results: Successful MCAO (involving whole MCA territories) was sustained at 55 minutes in 7 of 33 rats. Only H&E staining defined boundaries between normal tissue and ischemic pallor areas grossly, consistent with abnormal ADC lesions in all 7 animals. High power light microscopy (× 200, × 400) confirmed presence of picknotic neurons and vacuolated astrocytes in the H&E pallor areas. Other staining methods failed to diagnose focal ischemic areas on both gross and microscopic pathology.
Conclusion: At 1hour after permanent MCAO in rats, hyperacute focal cerebral ischemia involving whole MCA territories was identified by H&E gross and light microscopic pathology and correlated with DWI .



[ADC image and pathology (Cresyl violet, MAP2, H&E)]

PS06-034

Poster Viewing Session VI

Role of NADPH oxidase in mitochondrial dysfunction following transient global cerebral ischemia in rats

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Abstract
Cerebral ischemia results from occlusion of major arteries of brain and reactive oxygen species play major role in its pathology. Major sources of ROS are NADPH oxidase (PHOX) and mitochondria and they both are interlinked.

Activation of one enzyme system lead to activation of other, resulting in oxidative stress which lead to neurodegeneration. Present study tried to explore the role of PHOX inhibitor to attenuate the mitochondrial injury following ischemia in rats. Ischemia model established by occlusion of both common carotid arteries for 15 minutes. Mitochondrial membrane potential (ψm) and activity of mitochondrial complexes were studied. Apoptosis were studied by TUNEL assay and gene expression of various markers cyt c, Bcl2, Bax, caspase3 and 9 were seen. Neurodegeneration were observed by fluorojade staining. PHOX activity and ROS levels were significantly increased post 7 days of ischemic injury. Activity of mitochondrial complexes I, II, IV, V were significantly decreased following ischemia. Consequently there was decrease in mitochondrial membrane potential that lead to change in membrane transition pore. Increased cytochrome c and other apoptotic genes initiated the programmed cell death which was reflected by TUNEL positive cells and Flourojade B positive cells in cortical and hippocampal region. The administration of apocynin significantly reduce the PHOX activity and hence ROS production which result in decreased mitochondrial injury and apoptosis. Inhibition of NADPH oxidase activity is a therapeutic target to attenuate the mitochondrial injury following ischemia.

PS06-035

Poster Viewing Session VI

Characterisation of Translocator Protein (TSPO) expression in the brain in rodent models of neuroinflammation

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Abstract

Mitochondrial membrane Translocator Protein (TSPO) is implicated in many physiological functions including steroidogenesis, stress sensing, autophagy and cholesterol transport, but its exact roles remain to be elucidated(1). Despite the uncertainty, a significant interest in TSPO is based on the ability to detect its expression *in vivo* by PET, via the use of radiolabelled ligands. It appears that TSPO expression is increased during inflammation in the CNS and thus TSPO ligand-binding is a useful biomarker of neuroinflammation in psychiatric and neurological research. It is not yet clear whether TSPO increase is linked to early acute neuroinflammation(2), a later 'inflammation-resolving phase'(3) or whether it is preferentially shown in the prolonged chronic neuroinflammation.

In order to examine which type of inflammatory processes affect TSPO, we measured TSPO expression in several rat models of neuroinflammation: systemic acute administration of bacterial endotoxin lipopolysaccharide (LPS), systemic chronic administration of LPS, intracerebral administration of LPS and photothrombotic stroke in the sensorimotor cortex. All these models demonstrate a prominent and persistent activation and proliferation of microglia, the brain's resident immune cells.

Despite evidence of microglial activation (immunohistochemistry), we did not detect increased TSPO binding (3H-PK11195 autoradiography) in the brains of rats treated with a single systemic dose of LPS. TSPO was clearly increased in stroke, and its time-course matched that of microglia proliferation (but not activation), whereas in the model of intracerebral administration TSPO increase was immediately present. We further explored the origin and source of TSPO expression, by conducting analyses of blood brain barrier permeability and immunohistochemical co-localization studies. We present a picture of TSPO in neuroinflammation that is complex and dependant on the nature and timing of the insult.

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PS06-036

Poster Viewing Session VI

Microglial calcium release-activated calcium (CRAC) channel inhibition improves outcome from experimental traumatic brain injury and microglia-induced neuronal death

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Abstract

Store-operated Ca^{2+} entry (SOCE) mediated by CRAC channels contribute to calcium signaling in immune cells. CRAC channels consist of the endoplasmic reticulum resident Ca^{2+}-sensing protein stromal interaction molecule 1 (STIM1) and the calcium channel protein ORAI1 located in the plasma membrane. Prolonged Ca^{2+} entry through CRAC channels activates nuclear factor of activated T cells (NFAT), involved in T cell proliferation and cytokine expression. Microglia contain CRAC channels, but little is known whether they play a role in brain injury. We studied novel CRAC channel inhibitors to explore their therapeutic potential in microglia-mediated injury. A neuron cell line (Neuro-2A, N-2A) was cultured alone or with microglial BV2 cells then exposed to 2h oxygen glucose deprivation (OGD). Some cultures were treated with a novel CRAC channel inhibitor. Toll-like receptor (TLR) -3, -4 agonists or IFNγ were also used to activate microglia. Western blots revealed the presence of CRAC channel proteins STIM1 and ORAI1 in microglia. CRAC channel inhibition decreased NO release and inflammatory proteins iNOS and COX-2 expression in activated microglia, but did not affect STIM1 or ORAI1 expression. CRAC channel inhibitors also reduced agonist induced intracellular calcium accumulation in BV2 cells. Agonists also activated JNK1/2 kinase, NFAT, NF-kB, CREB & STAT1 in microglia, but only JNK1/2 kinase & NFAT were attenuated by inhibitor. OGD decreased N2A neuronal cell viability, further exacerbated by BV2 cells, but neuronal cells were protected by CRAC channel inhibition ($n = 5$, $^*p < 0.05$). We then treated male C57/BL6 mice exposed to experimental brain trauma (TBI) and found that CRAC channel inhibition led to decreased lesion size, brain hemorrhage and improved neurological deficits ($n = 6$–7/grp, $^*p < 0.05$). We suggest a novel anti-inflammatory approach for treating acute brain injury. Our observations also shed light on new calcium signaling pathways, not previously described in brain injury models.

PS06-037

Poster Viewing Session VI

Triggering receptor expressed on myeloid cells-2 (TREM2) biases towards M2 microglial responses and its deficiency worsens outcome after experimental traumatic brain injury

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Abstract

Triggering receptor expressed by myeloid cells-2 (TREM2) is a surface receptor present on microglia and macrophages. It was first described as a receptor of the innate immune system which bound pathogens and led to their phagocytosis. TREM2 deficiency leads to dementia, and we recently showed that its deficiency leads to worsened outcome after experimental stroke. Thus, TREM2 may be an important molecule in the clearance of injured cells paving the way towards recovery and repair. Here, we explored whether TREM2 might also play a role in experimental brain trauma TBI. Male TREM2 knockout (TREM Ko) or wildtype (Wt) mice ($n = 12$/group) were subjected to controlled cortical impact (CCI), then assessed for neurological function at 1, 3, 7 & 14 d later. Mice were assessed for neurological deficit (Bederson score), lateralization on an elevated body swing test, and foot faults from a ladder test. Brains were then harvested for histology. TREM Ko mice had worsened neurological recovery compared to Wt on all three functional studies ($p < 0.05$) and had markedly increased lesion volumes ($2\times$ larger than

Wt, $p < 0.01$). This was also associated with reduced resorption of injured brain tissue amongst Ko mice. We also studied Neuro2a cells in combination with BV2 microglia. Microglial treatment with IL-4 induced a M2 (anti-inflammatory) phenotype as determined by arginase 1, CD206 & YM1 induction, and also increased TREM2 expression. When TREM2 was silenced using siRNA, arginase1 induction was decreased while iNOS (M1, pro-inflammatory) was increased. TREM2 silencing in BV2 cells also decreased neuronal phagocytosis in response to microglial activators. These results indicate that TREM2 deficiency worsens outcome from experimental TBI, and that TREM2 plays a role in the phagocytosis of injured brain cells, and leads towards a M2 phenotype.

PS06-038

Poster Viewing Session VI

The potential neuroprotective role of a histone deacetylase inhibitor, sodium butyrate, after neonatal hypoxia-ischemia

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Abstract

Background: Histone deacetylase inhibitor (HDACi) - sodium butyrate (SB) has been shown to be neuroprotective in adult brain injury models. Potential explanation for the inhibitor action involves among others reduced inflammation. The aim of our study was to test the hypothesis that one of the supposed mechanisms of protection afforded by SB after neonatal hypoxia-ischemia may be also associated with anti-inflammatory action. We examined the effect of SB on the production of inflammatory factors including analysis of the microglial and astrocytic cell response. We also examined the effect of SB on molecular mediators that are crucial for inducing cerebral damage after ischemia (transcription factors, pro- and anti-apoptotic proteins).

Methods: Seven-day-old rat pups were subjected to unilateral carotid artery ligation followed by 60 minutes of hypoxia (7.6% O_2). SB (300 mg/kg) was administered in a 5-day regime with the first injection given immediately after hypoxic exposure. The damage of the ipsilateral hemisphere was evaluated by hematoxylin-eosin staining (HE). Microglial and astroglial cells were identified by immunohistochemistry. Effects of SB on HI-induced inflammation (cytokines and chemokine) were assessed by Luminex assay. Expression of molecular mediators (NFκB, p53, COX-2, Bax, Bcl-2,) were assayed by Western blot.

Results: SB treatment reduced brain damage, as assessed by HE staining, suppressed the production of inflammatory markers - IL-1β, chemokine CXCL10 and blocked ischemia-elicited up-regulation of COX-2 in the damaged ipsilateral hemisphere. Furthermore, administration of SB promoted the conversion of microglia phenotype from inflammatory M1 to anti-inflammatory M2. None of the investigated molecular mediators that are known to be affected by HDACis in adults were modified after SB administration.

Conclusions: SB appears to exert a beneficial effect in neonatal hypoxia-ischemia injury via suppression of HI-induced cerebral inflammation.

Supported by National Science Centre, Poland grants: 2012/05/B/NZ3/00436, 2014/15/B/NZ4/01875 and Mossakowski Medical Research Centre Statutory Fund no. 17/2016.

PS06-039

Poster Viewing Session VI

Role of DAMPs-mediated NETosis during the acute inflammation in the postischemic brain

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Abstract

Cerebral ischemia leads to brain damages via a complicated pathological events such as excitotoxicity, inflammation, apoptosis, and peri-infarct depolarization. Brain inflammation after stroke is known to be slowly proceeded with low impacts on forming brain infarction However, neutrophils are quickly recruited in response to brain damages and the role of neutrophils is not fully understood after recruitment. We investigated recruitment of neutrophils and formation of neutrophil extracellular traps (NETs) in permanent middle cerebral artery occlusion

(pMCAO) model. H&E and immunohistochemistry identified the recruitment of neutrophil in leptomeningeal artery and corpus callosum after pMCAO. Immunofluorescence staining revealed that the number of CitH3 (NETosis marker) positive cells, which showed intact, partial lytic, or lytic, increased in leptomeninges from 6 hours, in cortex from 12 hours, and in striatum from 24 hours after pMCAO. At 24 hours after pMCAO, lytic CitH3 positive cells prevailed in cortical arteriole and microvessel, which supposed to induce vascular damage and subsequent infiltration of immune cells. Furthermore, neutrophils were also recruited in cerebrospinal fluid (CSF) after pMCAO, which identified CitH3 positive cells. Interestingly, neutrophils in CSF and corpus callosum after pMCAO were shrunken, compared with blood neutrophils. Because cerebral ischemia is non-infectious disease, we assumed danger associated molecular patterns (DAMPs) stimulated neutrophils to migrate to damaged brain parenchyma and form NET in the postischemic brain. The level of CitH3 and NET formation were increased by treatment of HMGB1, glutamate, or ATP, which were suppressed by respective antagonist. Together these results indicated that the recruitment of neutrophils and NET formation in the postischemic brain were regulated by DAMPs and damaged vessel by NETosis accelerated subsequent recruitment of other immune cells, followed by brain inflammation.

PS06-040

Poster Viewing Session VI

Immunomodulatory properties of human bone marrow mesenchymal stem cells and extracellular vesicles derived from these cells after their transplantation into focal brain ischemic rats

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Abstract

Introduction: Mesenchymal stem cells (MSCs) are a potential tool for cell-based therapies in regenerative medicine. The chief therapeutic attributes of MSCs seems to be their capacity to promote functional recovery of damaged tissues and modulate immune responses. Recent studies suggest that therapeutic functions of MSCs are linked to the production of extracellular vesicles.

Aim: The aim of the study was to compare the immunomodulatory properties of human bone marrow mesenchymal stem cells (hBM-MSCs) and extracellular vesicles (EVs) derived from these cells in focal brain ischemic rats.

Methods: The experiments were performed in adult male Wistar rats with focal brain injury of 1 μl/50nmol ouabain injected into the right hemisphere. Two days after the brain insult, 5×105 hBM-MSCs (Lonza) labelled with iron nanoparticles conjugated with rhodamine (Molday, BioPAL) or EVs derived from 5×106 hBM-MSCs stained with PKH26 (Sigma) were transplanted into the right internal carotid artery. The inflow of transplanted hBM-MSCs and EVs was monitored using MRI and the presence of the donor cells or their derives in the rat brain was confirmed by confocal microscopy analysis. The immunohistochemical analysis of immunological response in brain, cervical lymph nodes and spleen after hBM-MSCs or EVs transplantation was performed.

Results and Conclusions: Our studies revealed that hBM-MSCs and EVs injected intra-arterially migrated into the rat brain and were visible in MRI in the right hemisphere up to 7 days after transplantation which was confirmed by immunohistochemical analysis. The decrease of activated astrocytes ($GFAP^+$), microglia ($ED1^+$) and leukocytes ($CD45RA^+$), including neutrophils ($CD15^+$) and the increase of lymphocytes T ($CD5^+$)was observed in the injured brain tissue after hBM-MSCs injection.

Supported by KNOW 06 project: "The role of bone marrow mesenchymal stem cells and microvesicles derived from these cells in CNS repair of brain ischemia disorder" and MMRC statutory grant no 17.

PS06-041

Poster Viewing Session VI

Changes in local and global measures of resting state functional connectivity in the rat LPS model of neuroinflammation

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Abstract

Lipopolysaccharide (LPS) causes a generalized immune reaction, which also affects the CNS. The resulting sickness behaviour, characterized by a loss of appetite and hypolocomotion, is linked to depressive illness and efforts are made to understand how the neuroinflammation causes behavioural symptoms. Here we aimed to characterize the brain connectivity in rats treated with LPS, in order to develop *in vivo* translatable biomarker of neuroinflammation.

Male SD rats were imaged by resting state functional MRI (rsfMRI) before, 24 hr and 2 weeks after 0.5 mg/kg ip LPS ($n = 18$) or saline (VEH, $n = 19$). All LPS treated rats showed expected sickness behaviour and weight loss (+0.5 g VEH, -25 g LPS, $p < 0.0001$) at 24 h; widespread neuroinflammation in this model (inflammatory cytokines and activated microglia in the brain) was confirmed in separate cohorts of rats. rsfMRI data were pre-processed using FSL's FEAT and FIX tools, converted into graph networks (154 ROIs) and statistically analyzed for changes in graph theoretical measures using GRETNA1 and NBS2.

First, the presences of robust homotopic connectivity networks were assessed in each animal to validate the methodology, using ICA (A). Then, LPS and VEH networks were compared at the global, nodal and edge level at each time point. Differences were detected at 24 hr, but not at 2 weeks, after treatment.

We observed changes in localized network properties including higher modularity and increased nodal clustering coefficients in LPS rats, which may suggest a more fragmented network in the inflamed brain due to transient attenuation of existing connections. The hotspot areas for decreased connectivity were subcortical, particularly the thalamus. Moreover we found significantly strengthened and attenuated edge connections between several regions in LPS rats (B).

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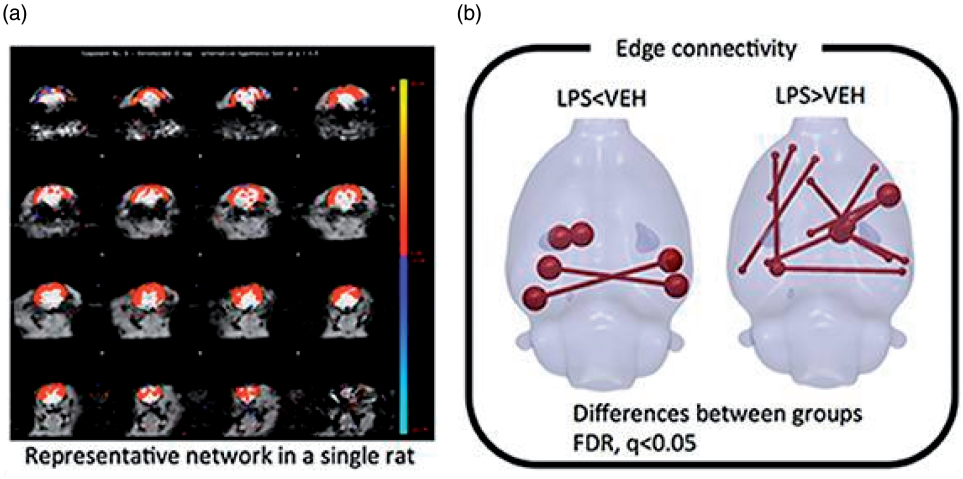
Figure



PS06-042

Poster Viewing Session VI

The role of pentraxin 3 (PTX3) in inflammation and neuroprotection after ischaemic stroke

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Abstract
The inflammatory response occurring after an ischaemic stroke is a key mediator of stroke pathogenesis. Thus, targeting inflammation and promoting repair mechanisms are attractive therapeutic approaches for stroke. A recent study has shown the acute phase protein pentraxin 3 (PTX3) as potentially neuroprotective as it reduces blood brain barrier (BBB) damage and promotes resolution of oedema, angiogenesis and neurogenesis after experimental stroke. Our aim was to investigate this further, confirming neuroprotective actions of PTX3 *in vitro*, and identifying possible underlying mechanisms by studying PTX3 recruitment of neutrophils.
Neurones were treated with 20 μM N-methyl-D-aspartate (NMDA), PTX3 (1000 ng/ml) or 20 μM NMDA and PTX3 (1000 ng/ml). In separate studies, neutrophils were isolated from wild type (WT) and PTX3 knock out (KO) mice and allowed to transmigrate through interleukin-1 beta (IL1β) activated brain endothelial (bEnd5) cells for 24 h. Subsequently, neutrophil transmigration was quantified with a cell haemocytometer. Naive or transmigrated WT and KO neutrophils were seeded onto primary mouse cortical neurones and incubated for 20 h. In all studies, neuronal cell death was quantified with lactate dehydrogenase (LDH) assay.
We found *in vitro* that neuronal cell death induced by NMDA was significantly reduced 3 fold in cultures treated with a combination of NMDA (20 μM) and PTX3 (1000 ng/ml) compared with cultures treated with NMDA (20 μM) alone. We found that neutrophil transmigration was significantly reduced 3 fold in PTX3 KO mice compared with WT controls. Naïve WT and KO neutrophils were significantly less neurotoxic than transmigrated WT and KO neutrophils, however, no significant difference in neurotoxicity was observed between WT and KO transmigrated neutrophils.
These data suggest that PTX3 has neuroprotective properties and may also play a role in inflammation by regulating neutrophil transmigration. Our findings suggest that PTX3 is a promising therapeutic target for future stroke therapies.

PS06-043

Poster Viewing Session VI

A translational model of moderate perinatal asphyxia reveals lasting behavioral deficits in the absence of focal neuronal loss

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Abstract
Epidemiological studies suggest that even moderate birth asphyxia may play a role in the development of psychiatric disorders. Our goal was to characterize a recently developed translational rodent model of perinatal asphyxia which does not include any surgical intervention, in order to investigate putative mechanisms through which asphyxia may contribute to the subsequent behavioral alterations.
Wistar rat pups on postnatal day 7 were exposed to a gas mixture containing 4% O_2, 20% CO_2 and 76% N_2, or to room air for 15 min at 37 °C. Rats were subjected to comprehensive behavioral assessment from 24 h post-asphyxia into adulthood. Brain perfusion and microglial activation were investigated with SPECT and MRI in vivo. Inflammation and neuronal injury were studied by using

cytometric bead array, immunofluorescence and confocal/super-resolution microscopy.
Brain perfusion changes, microglial activation and neuronal apoptosis were detected 24 h after asphyxia. In adult rats, changes in vesicular glutamate transporter (VGLUT-2) levels in synaptic terminals were found in the CA1-CA3 regions of the hippocampus and in the prefrontal cortex, whereas no signs of focal neuronal loss or white matter injury were observed. Neonatal asphyxia did not cause significant sensory-motor deficits in pups or in adult rats. However, long-term functional testing identified increased anxiety of asphyxiated rats in the Elevated Plus Maze and spatial memory deficits were found in the Morris water maze test. Moreover, changes in emotional and memory functions in adult post-asphyxia rats were accompanied by increased impulsivity-like behavior and attention deficits in the Delay Discounting paradigm and in the 5-choice test.
Moderate neonatal asphyxia evoked acute neuroinflammation in the absence of major neuronal injury and resulted in dysfunction of glutamatergic terminals in conjunction with ADHD-like behavioral symptoms in adulthood. This model may provide clinically relevant insight into mechanisms of the diverse psychiatric disturbances in humans who have suffered from moderate birth asphyxia.

PS06-044

Poster Viewing Session VI

Phosphoinositide 3-kinase χ ties chemoattractant- and adrenergic control of microglial motility

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Abstract
Microglial motility is tightly controlled by multitude of agonistic and antagonistic factors. Chemoattractants, released after brain infection or damage, provoke directed migration of microglia to the pathogenic incident. In contrast, noradrenaline and other stress hormones have been shown to suppress microglial movement. Here we asked for the signaling reactions involved in the balanced control of microglial motility.
Using pharmacological and genetic approaches we identified the lipid kinase activity of phosphoinositide 3-kinase species χ (PI3Kχ) as an essential mediator of microglial migration. We verified with appropriate in vitro assays using immortalized and primary microglial cells that C5a acts as most effective inducer of microglial migration. The PI3Kχ dependence of Iba1-positive cell motility was confirmed under *in vivo* conditions. We could shown that Iba1-positive cell migration in direction of a focal stab injury revealed reduced cell number in the immediate vicinity of the injury site indicating reduced directed migration in PI3Kχ knockout brains. Furthermore, noradrenaline induced cAMP production and subsequent stimulation of protein kinase A (PKA) was verified as inhibitory principle of microglial migration. Decrease of microglial migration after norepinephrine stimulation could be rescued by co-treatment with the PKA inhibitor H89.
Therefore, inhibition of PI3Kχ lipid kinase activity by protein kinase A was disclosed as mechanism causing suppression of microglial migration by noradrenaline. Together these data characterize PI3Kχ as a nodal point in the control of microglial motility.

PS06-045

Poster Viewing Session VI

Characteristics of primary rat microglia isolated from mixed cultures using two different methods

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Abstract
Objectives: Microglia are resident immune cells of the brain. Any insult to the CNS causes microglial activation. Activated microglia undergo morphological and functional changes. Mild trypsinization and shaking are two of the most commonly used methods to isolate microglia from mixed cultures. In this study, we compared the characteristics of primary cultured rat microglia obtained using these two methods both under normal condition and after various stimuli.
Methods: Primary rat microglia cultures were prepared from cerebral cortices of 1-2-day-old neonatal

Sprague-Dawley rats. After 14 days in vitro, microglia were isolated from mixed glial cultures via either mild trypsinization or shaking. The purity of microglia was estimated by flow cytometry. Quantitative gene expression was measured using real-time PCR. Levels of cytokines in cell culture supernatant were measured using ELISA. Microglia Phagocytosis was assessed with *Escherichia coli* K-12 BioParticles.

Results: Mild trypsinization generated higher yield and purity than shaking. Compared with shaking, microglia isolated by mild trypsinization were in a quiescent state under normal condition, showing uniform ramified morphology, lower baseline levels of gene expression, cytokines release, and phagocytic capability. Microglia isolated by shaking showed "ameboid" morphology. In contrast, microglia obtained by either mild trypsinization or shaking were fully functional. The response patterns of gene expression (iNOS, CD86, CD206, Arginase 1, CX3CR1, TLR2, and CCR2) and cytokines release (TNFα, IL-1b, IL-10, and IGF-1) to various stimuli (IL-4, LPS, or IFNγ) were similar in microglia isolated by both methods.

Conclusions: Mild trypsinization is a reliable method to isolate microglia from mixed glia cultures with superior yield and purity. Microglia purified by mild trypsinization were closer to their "resting" state, whereas microglia isolated by shaking were in an activated state. It should be carefully considered when choosing the culture method for microglia studies since microglia were sensitive and the state of microglia was influenced by the culture method.

PS06-046

Poster Viewing Session VI

Contribution of inflammasomes to ischaemic stroke

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Abstract

Objectives: Inflammation plays a key role across the time course of stroke and is known to exacerbate brain damage during the acute phase. Several regulatory molecules are implicated in inflammation, but the most established inflammatory mediator of acute brain injury is the cytokine interleukin-1. Interleukin-1 is regulated by large, multimolecular complexes called inflammasomes. Here, we investigate the expression of inflammasomes after stroke.

Methods: To induce stroke, we used an *in vivo* model of middle cerebral artery thrombosis through topical application of $FeCl_3$ in mice. Using qPCR and immunofluorescence, we investigated central and systemic inflammasome expression at both the transcriptional and translational level.

Results: We observed an increase in gene and protein expression of NLRP3 both centrally, in the ipsilateral cortex, and systemically, within the bone marrow, 24 h after stroke. Other inflammasomes did not change.

Conclusions: These data suggest NLRP3 contributes to the central and systemic inflammatory response after stroke. Further experiments with NLRP3$^{-/-}$ mice will clarify the role of NLRP3 in the communication between the central and systemic inflammatory responses to stroke.

PS06-047

Poster Viewing Session VI

Telomere maintenance, mitochondrial biogenesis and cell cycle control: an mRNA study of microglia activation states

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Abstract

Objectives: Microglia senescence may promote neuropsychiatric disease. This prompted us to examine the relationship between microglia activation states and telomere biology.

Methods: A panel of candidate genes associated with telomere maintenance, mitochondrial biogenesis, and cell cycle regulation were investigated in M1- and M2-polarized microglia *in vitro* as well as in MACS-purified CD11b+

microglia/brain macrophages from models of stroke, Alzheimer's disease, and chronic stress.
Results: M1 polarization, ischemia, and Alzheimer pathology elicited a strikingly similar transcriptomic profile with, in particular, reduced expression of murine *Tert*.
Conclusions: Our results link classical microglia activation with repression of telomere-associated genes, suggesting a new mechanism underlying microglia dysfunction.

PS06-048

Poster Viewing Session VI

Reactive oxygen species (ROS)-responsive nanotechnology: Therapeutic action of nanoparticles in stroke

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Abstract
Ischaemic stroke is characterised by a potent inflammatory response that contributes to brain injury. Inflammatory responses are associated with oxidative stress, production of highly oxidising compounds, such as superoxide anion, hydrogen peroxide and hypochlorite (all of which are reactive oxygen species (ROS)). There is evidence to suggest that immediately after acute ischaemic stroke there is a rapid increase in the production of ROS, which have both directly toxic and inflammatory effects. Therefore, one way to protect the brain after ischaemic stroke is by blocking the actions of ROS in order to reduce inflammation. Recently, oxidation-responsive poly(propylene sulphide (PPS) nanoparticles, that predominantly scavenge upregulated concentrations of extracellular ROS, have been developed. Therefore, we tested the hypothesis that these nanoparticles are effective at scavenging ROS produced by cells of the brain, and can therefore limit inflammation and protect the brain tissue after ischaemic stroke. Primary mouse mixed glial cells (astrocytes and microglia) were treated with 0.5 μg/ml of inflammatory mediator lipopolysaccharide (LPS). Poly(propylene sulphide) (PPS) nanoparticles significantly decreased LPS-induced production of interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), detected with enzyme linked immunosorbent assay (ELISA). Similar results were obtained for the analysis of nitric oxide (NO) (in the form of nitrite). PPS nanoparticles significantly decreased nitrite production in immortalised murine microglial cell cultures (BV-2) treated with 1 μg/ml LPS, as detected with griess reagent system. Exposure of PPS nanoparticles (1–5 mg/ml) to 15 micromolar hydrogen peroxide (H_2O_2) for 24 hours significantly decreased H_2O_2 concentration in a concentration-dependent manner, detected with ROS-Glo^{TM} H_2O_2 Assay.
PPS nanoparticles caused a dose-dependent decrease of nitrite, H_2O_2, IL-6 and TNF-α production. These data suggest that PPS nanoparticles may be scavenging ROS, thus decreasing ROS stimulation of cells to produce inflammatory cytokines. Therefore, PPS nanoparticles can be considered as anti-oxidant and anti-neuroinflammatory therapy for ischaemic stroke.

PS06-049

Poster Viewing Session VI

Cerebral blood flow, optic nerve sheath diameter and neurological status evaluation in patients submitted to post-traumatic decompressive craniectomy and late cranioplasty

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Abstract
Background: Traumatic brain injury (TBI) is common worldwide and decompressive craniectomy (DC) may be a life-saving procedure in these patients. Besides the cosmetic and protective effect of cranioplasty (CP) after DC, recent studies have shown neurological, metabolical and cerebral blood flow improvement. Better results are described when cranial reconstruction is performed

early after DC. However, at the public health system in developing countries, the CP is usually carried-out later.
Objective: To verify the effect of late CP on cerebral hemodynamics and neurological functional recovery at a public tertiary trauma hospital in Brazil.
Methods: Patients who underwent large DC for severe TBI and were later submitted to CP were prospectively studied before and 3 months after cranial reconstruction. Hemodynamic evaluation was performed by ultrasonographic cerebral blood flow velocity (CBFV) and optic nerve sheath diameter (ONSD) measurements. Neurological status was evaluated by Mini Mental State Examination (MMSE), Barthel Index and Rankin Modified Score.
Results: 17 patients were studied; 16 were men (mean age = 30 yo). Mean time interval between DC and CP was 17 months. CBFV at the middle cerebral artery (MCA) was lower at the skull defect side(59.5 vs. 76.2 cm/s; $p = 0.01$). After CP, increases of CBFV at MCA ($p = 0.01$) and anterior cerebral artery ($p = 0.01$) at ipsilateral side were verified. OSND was larger at skull defect side (0.58 vs. 0.54 cm; $p = 0.14$) before CP and significantly reduced (0.58 vs. 0.53 cm; $p = 0.02$) after the procedure. There was also a positive correlation between CBFV increase at MCA and MMSE improvement performance ($p = 0.004$).
Conclusion: Cranioplasty can favorably modify cerebral hemodynamics and improve the neurological status of patients even if performed in a late phase. ONSD enlargement may reinforce the theory about the effect of atmospheric pressure upon a brain with skull defect in DC.

PS06-050

Poster Viewing Session VI

Sex differences in brain blood flow in rugby players with concussions

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Abstract
Increasing evidence is suggesting that concussions are a possible significant cause of long term cognitive and health problems among athletes. Despite this increasing evidence, there is a lack of data on the physiological response immediately following a head injury. The goal of this work was to determine if concussions cause impairment of cerebral blood flow regulation in the first few hours after injury and to determine if there is a sex difference in the response.
During several recreational rugby tournaments we recruited a total of 34 players. Of these players, 26 were recruited as controls (5 females) and 8 had suffered a concussion (3 females). All testing was performed on the field to assess cerebral blood flow using carotid ultrasonography, beat-by-beat blood pressure, and end-tidal CO_2 while supine. Data was collected 131 ± 23 min following the head trauma.
Concussed players demonstrated significantly greater mean arterial pressures regardless of sex (Males - Ctrl 74 ± 10 vs Concussed 79 ± 14; Females Ctrl 67 ± 7 vs Concussed 82 ± 14 mmHg, $P = 0.034$). In contrast, internal carotid blood flow was no different in males (Ctrl 623 ± 173 vs Concussed 619 ± 214 mL/min), but tended to be lower in concussed females (Ctrl 619 ± 214 vs Concussed 448 ± 95 mL/min). This reduced cerebral blood flow was also associated with an increase in cerebrovascular resistance in females (Ctrl 0.12 ± 0.04 vs Concussed 0.18 ± 0.01 mmHg/(mL/min)) that did not occur in males (Ctrl 0.13 ± 0.04 vs Concussed 0.14 ± 0.05 mmHg/(mL/min)).
These data suggest that while both males and females had similar hypertensive responses following a sports related concussion, a tendency towards a greater decrease in cerebral flow when standing was found in the females. Thus further work is necessary to both understand the physiological response following concussion, but specifically with regards to sex differences.
This work was supported by the WRIISC within the Department of Veteran Affairs.

PS06-051

Poster Viewing Session VI

The expression of NP847 and Sox2 after TBI and its influence on NSCs

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Abstract
The proliferation and differentiation of neural stem cells (NSCs) is important for neural regeneration after cerebral

injury. Here, for the first time, we show that phosphorylated (p)-ser847-nNOS (NP847), rather than nNOS, may play a major role in NSC proliferation after TBI. Western blot results demonstrated that the expression of NP847 and Sox2 in the hippocampus is up-regulated after TBI, and they both peak 3 days after brain injury. In addition, an immunofluorescence experiment indicated that NP847 and Sox2 partly co-localize in the nuclei of NSCs after TBI. Further immunoprecipitation experiments found that NP847 and Sox2 can directly interact with each other in NSCs. Moreover, in an OGD model of NSCs, NP847 expression is decreased, which is followed by the down-regulation of Sox2. Interestingly, in this study, we did not observe changes in the expression of nNOS in the OGD model. Further research data suggest that the NP847-Sox2 complex may play a major role in NSCs through the Shh/Gli signaling pathway in a CaMKII-dependent manner after brain injury.

PS06-052

Poster Viewing Session VI

Effects of pyrroloquinoline quinone on WISP1 in traumatic brain injury

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Abstract
WISP1, as a member of the CCN4 protein family, has cell protective effects of promoting cell proliferation and inhibiting cell apoptosis. Traumatic brain injury model was established to study the role of WISP1 in nervous system in rat. Results showed WISP1 mRNA expression decreased at 3 d, increased at 5 d after TBI. WISP1 protein expression was similar to the mRNA expression of WISP1. Meanwhile, immunofluorescence was used to detect the cell location of WISP1, which demonstrated that there was little of co-location about WISP1 with GFAP, Iba1 respectively. However, WISP1 co-localized with NeuN partly. Double-labeling immunofluorescence about WISP1 with LC3 suggested that WISP1 was involved in autophagy. But there was little of co-location about WISP1 with Cleaved-Caspase3, which suggested that WISP1 was not directly relative to apoptosis. Subsequent study displayed that the protein expression trend of β-catenin was identical to that of WISP1 after TBI. Immunofluorescence in PC12 or SHSY5Y cells showed that WISP1 maily located in cytoplasm. WISP1 expression reduced obviously in SHSY5Y cells after being transfected with WISP1 si-RNA compared with negative control group. The effects of different concentrations or time of PQQ on the activity of PC12 and SHSY5Y cells were performed with CCK-8 assay, which suggested that PQQ had little influence on viability. Finally, Cell cycle was detected in SHSY5Y cells treated with 50 μM PQQ for 24 h after transfection with WISP1 si-RNA 72 h or 48 h. Results showed that there was no significant diversity. We thought that WISP1 plays a protective role after traumatic brain injury in rat and this effect may be relative to autophagy caused by traumatic brain injury.

PS06-053

Poster Viewing Session VI

Vascular reactivity changes of the intracortical blood vessels after juvenile traumatic brain injury

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Abstract
Objectives: Vascular dysfunction is a hallmark of pediatric TBI and predicts poor outcome on the long-term. Vascular reactivity in intraparenchymal vessels has never been investigated in juvenile TBI (jTBI). We hypothesized that vascular reactivity impairment in the jTBI group is associated with long-term behavioral dysfunction.
Methods: We used a new model **CHILD**, **C**losed-**H**ead **I**njury with **L**ong-term **D**isorders, in juvenile mice. Injury was induced with an electromagnetic impactor (3 mm diameter, 3 mm depth, 3 m/s speed) in postnatal day-17 mice. Vascular reactivity of the intraparenchymal blood vessels was measured in acute ispilateral cortical slices at day-1, 3, 7 and 30 post-TBI using thromboxane A2 receptor agonist U46619 application (vasoconstrictor). Behavior evaluations were performed and evaluation of various markers of the neurovascular unit, thromboxane A2 receptor (TXA2R) and α-smooth muscle actin (α-SMA) by Western blot and immunohistochemistry.
Results: The vascular response was significantly altered at days 1 and 3 post-TBI. At day 1, the blood vessels in jTBI group exhibited higher constriction than those in sham group at 10 minutes after U46619 application (Table).

On the contrary, the cortical blood vessels showed significantly reduced response in jTBI group compared to sham at 15 minutes after U46619 application at day 3 post-TBI (Table). The vascular reactivity changes were not associated with changes in TXA2Rs and α-SMA expression in the ipsilateral cortex for those timepoints. In parallel to these changes, the jTBI animals showed behavioral deficits (anxiety) at 1 and 3 days post-TBI.

Conclusion: The intraparenchymal blood vessels in the ipsilateral cortex exhibited altered vascular response to the TXA2Rs agonist during the first week after jTBI. The absence of correct vascular reactivity might contribute to the behavioral deficits observed in these animals. Molecular and cellular mechanisms behind those changes are currently under investigation.

	% sham constriction - % jTBI constriction		
	10 min	15 min	20 min
1 day post-TBI	20,75 (*)	9,09 (ns)	4,68 (ns)
3 days post-TBI	-14,03 (ns)	-22,11 (**)	-9,02 (ns)

**- $p \leq 0.01$; * - $p \leq 0.05$; ns – $p > 0.05$

[Vascular reactivity with U46619 application]

PS06-054

Poster Viewing Session VI

D-dimer as a potential marker for structural damage to the brain parenchyma in traumatic brain injury

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Abstract

Objectives: The incidence of coagulopathy is higher in traumatic brain injury (TBI) compared to the other injury. TBI-associated coagulopathy is correlated to poor outcome, while its mechanism remains unclear. Heavy blood loss and the volume of fluid resuscitation result in trauma-associated coagulopathy, however, these factors may not be critical in TBI-associated coagulopathy. We assumed that the extent of damage to cerebral tissue in TBI is associated the severity of coagulopathy. The purpose of this study was to find the coagulation/fibrinolytic parameters correlated to the structural damage to the brain parenchyma in TBI.

Methods: A total of 118 surgical patients for traumatic acute subdural hematoma (ASDH) and acute epidural hematoma (AEDH) between January 2008 and April 2015 were retrospectively reviewed. Complete blood counts, and conventional coagulation tests obtained on arrival. We used univariate analysis to evaluate the influence of structural damage to the brain parenchyma.

Results: Forty two patients were diagnosed with AEDH, whereas 76 patients suffered from ASDH with or without cerebral contusion. The percentage of patients with favorable outcome in ASDH patients was only 25%, while the 78.6% of AEDH patients had favorable prognosis. The serum D-dimer level was significant higher in ASDH patients compared with AEDH patients, however, no significant difference between ASDH and AEDH patients were seen in platelet count, PT-INR, fibrinogen.

Conclusions: In contrast to AEDH, ASDH due to a ruptured bridging veins and/or cortical vessel and commonly co-exist with cerebral contusions. Therefore, the structural damage to the brain parenchyma in ASDH was thought to be heavier than that in AEDH. Our results demonstrated that the serum D-dimer level was significant higher in ASDH patients compared with AEDH patient. Therefore, the serum d-dimer level may be a potential marker for structural damage to the brain parenchyma in TBI.

PS06-055

Poster Viewing Session VI

Evaluation of regional white matter volume reduction after diffuse axonal injury using voxel-based morphometry

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Abstract

Objectives: We developed a new and convenient method that employs voxel-based morphometry (VBM) to evaluate regional reduction in the volume of white matter after diffuse axonal injury (DAI).

Methods: We studied 29 patients with moderate cognitive disability after DAI. Each subject underwent 3-dimensional volumetric magnetic resonance (MR) imaging. Images were preprocessed automatically using standalone software running on a Windows PC for VBM of volumetric MR imaging utilizing a statistical parametric mapping (SPM) version 8 software engine and an algorithm for diffeomorphic anatomic registration through exponentiated Lie algebra (DARTEL). We then computed a Z-score for all coordinates on the white matter, which represented the relative reduction in white matter volume. Finally, we used voxel-based stereotactic extraction estimation (vbSEE)[1] to compute the extent of regional reduction in the volume of white matter (rWMVR) for each region of interest (ROI), defined as the rate of coordinates with Z-scores exceeding 2.0 in the ROI. For each ROI, we used Pearson's correlation analysis to examine the correlation between the extent of regional volume reduction and patient scores on the Wechsler Adult Intelligence Scale III (WAIS-III).
Results: We detected marked rWMVR in several ROIs, including the corpus callosum, and rWMVR correlated significantly with performance IQ and processing speed index in the splenium of the corpus callosum.
Conclusions: The results indicate the utility of our applications for the daily clinical evaluation of DAI. That they can be used on a PC and allow acquisition of volumetric data from standard MR images are their advantages.

References:

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PS06-056

Poster Viewing Session VI

Evaluation of neutrophil elastase (NE) as an early prediction marker of trauma induced disseminated intra vascular coagulation (DIC)

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Abstract
Background: Neutrophil elastase has a role in development of DIC after trauma. Failure to regulate the catalytic activity of this serine protease is the pathobiology of DIC. We aimed to investigate the prognostic significance of early measurement of neutrophil elastase in predicting DIC following injury.
Material and Method: A Prospective cohort analysis of 100 acutely injured trauma patients (70% TBI), (18 - 50 years) for one year. Patients with already existing coagulation abnormalities were excluded. ISTH DIC score was calculated. $\geq$5: overt DIC, 1–4: Non-overt DIC. Neutrophil elastase was measured within 24 hours of injury. DIC scoring and clinical follow up was done on 1st, 3rd and 5th day.
Results: 100 acutely injured patients were included in the study, 75 of them had Traumatic brain injury.
On admission overt DIC was diagnosed in 16 patients out of whom 11 died and 5 regressed to non overt DIC. On admission 77 had non-overt DIC: 23 died, 5 progressed to overt DIC, 41 remained non-overt DIC and 8 returned to normal. On admission 7 had no DIC: 2 died and 4 progressed to non-overt DIC.
Admission day elastase levels were significantly associated with the progression of DIC on day 5, with ROC of 0.63, a cut off of 1342 ng/μl was established (sensitivity of 71.4; specificity 43.1). 21% developed sepsis, 31% coagulopathy, and mortality was 36%. Development of overt DIC significantly correlated with thrombocytopenia and coagulopathy ($p = 0.02; < 0.001$ respectively), but not with sepsis and mortality. Plasma neutrophil elastase levels significantly varied between patients who died within 24 hr following trauma and patients who died after $\geq$72 hrs($p = 0.05$) and not with coagulopathy and sepsis ($p = 0.19$; 0.50 respectively).
Conclusion: A plasma NE level of 1342 ng/μl on admission would predict the development of DIC and early mortality following trauma.

PS06-057

Poster Viewing Session VI

Rolipram, a PDE-IV inhibitor protects against experimental Parkinsonism in mice

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Abstract

Objectives: Rolipram, a specific inhibitor of the phosphodiesterase IV (PDE IV), has recently been shown to exert neuroprotective effects in an Alzheimer transgenic mouse model and in hypoxic-ischemic damage in the rat brain. The cAMP mediated signaling is regulated by the activity of cyclic nucleotide phosphodiesterases (PDE) that cleave the second messenger. In the present study, we tested neuroprotective effects, if any, of rolipram drug, a specific inhibitor of the phosphodiesterase IV in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in mice.

Methods: Experimental animal is muscular weighing 25–30 g of 4-5-month-old. The drug was given four times at 12 h intervals by gavation (25-100 mg/kg) in animals made parkinsonian following two doses of MPTP (30 mg/kg, i.p.). Control mice were injected with the same volume of pure DMSO. MPTP-induced striatal dopamine depletion was significantly attenuated by higher dose of rolipram. MPTP-induced catalepsy and akinesia, as well as loss in swim ability, were blocked dose-dependently by rolipram. Brain was used for biochemical and histopathological study.

Results: Present study further shows that rolipram can dose-dependently attenuate both in vitro hydroxyl radical production in a Fenton-like reaction, and also ex vivo 1-methyl-4-phenylpyridinium (MPP+)-induced hydroxyl radical generation in isolated mitochondria. These results indicate that the observed neuroprotective effects of rolipram stem from its significant antioxidant action.

Conclusions: The preliminary results suggest that rolipram is a neuroprotector, and mechanism other than lipid lowering action could be the basis of this effect. Present data show a neuroprotective effect of the PDE IV specific inhibitor rolipram against dopaminergic neuron degeneration, suggesting that PDE IV inhibitors might be a potential treatment for Parkinson's disease.

PS06-058

Poster Viewing Session VI

Galectin-1-induced cognitive improvement, lower vascular and parenchymal amyloid deposition and immunomodulation in an animal model of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. Amyloid deposition and neuroinflammation are recognized hallmarks in AD, affecting mainly the brain cortex and hippocampus, both in patients and animal models. Galectin-1 (Gal1) -a glycan binding protein- is proposed to modulate several properties on immune and endothelial cells. Rabinovich's group (1) has previously reported a Gal1 neuroprotective role through deactivation of microglia in experimental autoimmune encephalitis, inducing an alternative phenotype. Here, we administered Gal1 or vehicle (i.p. 9 injections of 100ug/dose) during 3 weeks to 12-month-old PDAPPJ20 transgenic mice (Tg), or age-matched non-transgenic animals. Gal1 treatment improved the performance in the novel object location recognition test ($p < 0.05$). Congo red amyloid+ area in the hippocampus was decreased by 50% ($p < 0.05$) affecting mainly the dorsal hippocampus. Iba1+ microglial cells in the dentate gyrus exhibited less reactivity measured as soma size after Gal1 treatment ($p < 0.05$) while morphological score of microglial activation was significantly reduced, suggesting a modulatory effect on this cell population involved in amyloid clearance through phagocytosis. Moreover, the perivascular amyloid deposition decreased in treated Tg mice, visualized by tomato lectin labeling for microvasculature combined with Aβ immunofluorescence. In addition, we found that GFAP+ astrocytes surrounding amyloid plaques were Gal1+ in the hippocampus of Tg mice. We also generated PDAPPJ20/Gal1$^{-/-}$ animals, which exhibited an increased number of Iba1+ cells in

the hippocampus. Our results showed a potential relevant role for Gal1 in this neurodegenerative disease at multiple levels, including cellular and cognitive aspects. Additional *in vitro* experiments are in progress to identify the associated mechanism of action of Gal1 modulating the neuroinflammatory status in the context of AD.

Reference:

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PS06-061

Poster Viewing Session VI

Brain pericyte response in a mouse model of Huntington's disease

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Abstract

Background: The brain microvasculature has been of increasing interest in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and, more recently, Huntington's disease (HD) (1–3). In HD, vascular alteration comprises increased blood vessel density, blood brain barrier disruption and increased cerebral blood flow (3–4). While brain pericytes possess an important role in those features under physiological conditions (5), their response in the HD brain has yet to be elucidated.

Objectives: We examine the pericyte modulation in a mouse model of HD, at moderate and late stage of the disease.

Methods: We use a pericyte-specific reporter mouse where the *Green fluorescent protein* (*Gfp*) gene is expressed under the *Regulator for G-protein signaling 5* (*Rgs5*), thereby tracking activated pericytes, crossed with the R6/2 mouse model of HD.

Results: PDGFRβ^+ pericyte number increases in the R6/2 cortex and striatum, at mid and late stages of the disease. Moreover, brain pericytes upregulate RGS5 already from a moderate stage of the disease, which remains at late stage and is accompanied by the expression of another pericyte activation marker, Chondroitin sulphate proteoglycan NG2. Interestingly, we could not find any mutant Huntingtin aggregates in GFP$^+$ pericytes.

Conclusion: Our findings suggest that brain pericytes are activated in the HD brain. This may reflect a compensatory mechanism to stabilize the aberrant vasculature.

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PS06-062

Poster Viewing Session VI

Evaluation of CRISPr/SaCas9 induced gene knockdown in primary neurons and N27 cells

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Abstract

Latest advance in genome engineering methodologies based on the CRISPR/Cas9 have enabled researchers to interrogate the mammalian DNA in a precise and simple manner in any organism of choice. To better understand the role of the presynaptic compartment in Parkinson's disease, characterized by a progressive loss of dopaminergic neurons in the substantia nigra (SN), we used the CRISPR/Cas9 system to selectively target the vesicular monoamine transporter 2 (slc18a2) and the tyrosine hydroxylase (th) genes, involved in the storage, release and synthesis of dopamine in rats.

To selectively target dopaminergic cells, we used two double-floxed adeno-associated viral (AAV) vectors expressing the SaCas9 enzyme and the guide RNA (sgRNA) scaffolds. We designed five sgRNAs for each target gene and one control targeting the lacZ gene and tested their knockdown efficiency in primary cortical neurons and in a dopaminergic N27 cell line using a third AAV expressing Cre-recombinase. The knockdown efficiency of the sgRNAs was assessed on DNA (surveyor) and protein (immunofluorescence) level, one week post transduction. Following the *in vitro* selection, the best guides were

purified and injected together with the AAV-SaCas9 and AAV-Cre vectors at a concentration of 5.07×1014 gc/mL into the SN of naïve rats to determine possible toxicity effects. [11C]DTBZ PET experiments were performed 3 and 6weeks after AAV injections.
The *in vitro* data show, despite the low efficiency due to the three viral vector transduction, that two out of five sgRNAs for each target, were able to induce a selective knockdown of slc18a2 and th, if SaCas9, sgRNA and Cre were successfully co-expressed. *In vivo* PET experiments showed an initial decrease of the [11C]DTBZ BP_{ND} at the injected side at 3weeks, which returned to baseline levels 6weeks after injection. Next steps include the injection into DAT-Cre rats for a selective knockdown in dopaminergic neurons and functional imaging experiments.

PS06-063

Poster Viewing Session VI

Effects of L-DOPA and doxicycline administration on nociceptive responses in an animal model of Parkinson's disease

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Abstract
Parkinson's disease (PD) is a neurodegenerative disorder caused by progressive loss of catecholaminergic neurons, especially in the substantia nigra compacta (SNc), resulting in the loss of dopaminergic transmission in the nigrostriatal pathway motor. The pain is often observed in patients with PD, and this symptom often precedes the diagnosis of the disease. Recently, it has been recognized that 83% of patients with PD show changes in pain sensitivity. Little is known underlying mechanisms and pathophysiology of pain in patients with Parkinson's disease, however, animal models that reproduce the pathophysiology of this condition has been frequently used for the evaluation of involvement in symptoms in PD The usual applied in PD therapy is purely symptomatic relieving primarily in the restoration of the nigrostriatal function with the use of drugs that stimulate dopamine neurotransmission. L-dihydroxyphenylalanine (L-DOPA) is the most common strategy in the treatment of PD. We investigated thermal and mechanical nociceptive responses of rats with 6OHDA lesion in dopaminergic neurons in the medial forebrain bundle (FPM - nigrostriatal pathway). Further, the same tests were performed on rats chronically treated with L-DOPA. In addition, we evaluated the anti-inflammatory effect of doxycycline, in the thermal nociception test, before and after development of dyskinesia induced by L-DOPA.The results showed decreased nociceptive thresholds, in von Frey and hotplate tests after 6OHDA lesion and a reversion of this condition though L-DOPA at 30 mg/kg concentration. Also, doxicycline evidenced an major effect for attenuate the hypersensitivity condition after 14 days of treatment. This study provides first evidence that 6OHDA administration increases nociceptive responses in rats and indicate the potential role of dopaminergic mechanisms in the 6OHDA-induced nociception, since it was attenuated by L-DOPA treatment. Additionaly, neuroinflammatory mechanisms can be involved in this process, since doxycycline was effective for decrease nociceptive responses.

PS06-064

Poster Viewing Session VI

Middle cerebral artery pulsatility index as predictor for cognitive impairment in hypertensive patients

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Abstract
Objectives: Cognitive impairment as neurologic complications of hypertension related cerebrovascular disease has become global health issue due to increasing life expectancy. Gold standard diagnostic of vascular cognitive impairment (VCI) is through radiologic cerebral Magnetic Resonance Imaging (MRI). This study utilize another modality by using Transcranial Doppler (TCD) pulsatility index (PI) value of middle cerebral artery (MCA) to evaluate peripheral resistance. The purpose of this study was to determine if pulsatility index of MCA can be a predictor for cognitive impairment in hypertensive patients.
Methods: This is a cross sectional study conducted in Ciptomangunkusumo Hospital, Jakarta. Sixty six hypertensive subjects which lacked of macrovascular complications

were selected and screened using Montreal Cognitive Assesment-Indonesia version (MoCA-Ina) to determine their cognitive status. Scores ≥ 26 were grouped under cognitively normal subjects while scores ≤ 26 grouped under cognitive impairment subjects. Both groups then underwent TCD examination to determine values of PI MCA bilateral.

Results: Pulsatility index MCA were significantly higher in cognitive impairment group than normal group ($p < 0{,}001$). Subjects with elevated left side MCA PI has more tendency to suffer cognitive impairment rather than right side. Other confounding factors related to cognitive impairment would be ages and diabetes mellitus type 2 (DMT2).

Conclusion: Cognitive impairment hypertensive subjects has significantly higher PI MCA compared to cognitively normal hypertensive subjects. Pulsatility index of MCA can be a predictor for cognitive impairment in hypertensive subjects.

Keywords

Pulsatility index, middle cerebral artery, cognitive impairment, hypertension

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PS06-065

Poster Viewing Session VI

Capillary flow control in an aged model of Alzheimer's disease

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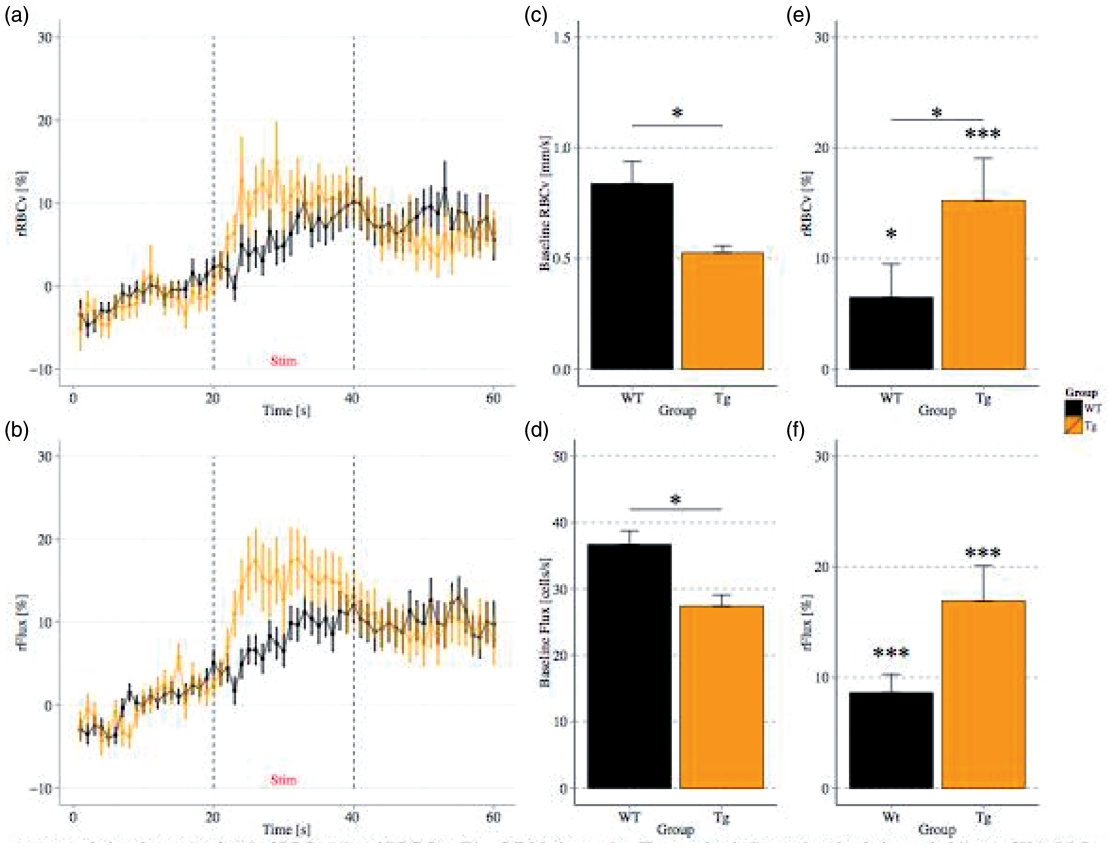
Abstract

Objectives: A hypothesis of the development of Alzheimer's disease (AD) states that capillary dysfunction produce an increase in capillary transit time heterogeneity (CTH). This wil require larger CBF response in order to maintain adequate oxygenation(1). This study aimed to investigate the potential role of changes in capillary flow patterns in AD.

Methods: We examined microvascular morphology and hemodynamics, including capillary flow homogenization in response to forepaw stimulation, in aged Tg-AD mice (APP^{SWE}/PS1ΔE9, $N = 9$) and their wild-type (WT, $N = 9$) littermates. We measured mean transit time (MTT), CTH and capillary hemodynamics using two-photon microscopy. We also analyzed the CTH:MTT ratio (CoV) that is expected to decrease during activation(2).

Results: Tg mice displayed lower capillary blood flow at rest than the WT mice (Tg: 27 ± 2 vs. 37 ± 3 cells/sec, $p < 0.001$). Capillary length density was similar in both groups. The hemodynamic response to stimulation is preserved in Tg mice and longer than in the WT mice ($p = 0.03$). Among the vascular units, activation only decreased CTH between arteriole and venule in the WT ($p = 0.031$).

Conclusions: We observed the Tg-AD mice showed low cortical perfusion at rest. Both groups had a similar capillary length density which indicated that maximal cortical oxygen delivery could be lower in Tg mice compared to WT mice. The augmented hemodynamic response to activation is still present in the Tg-AD mice. The reduction of CTH during activation previously observed in young mice(2), was absent in both groups. The aged WT mice showed a delay in the expected hemodynamic response during activation.



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PS06-066

Poster Viewing Session VI

Age drives the distortion of brain vascular, metabolic, and cognitive functions and the gut microbiome

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Abstract

Age is the top risk factor for the development of numerous diseases including neurological disorders such as dementia. Previous research has demonstrated brain vascular defects preceding dementia symptoms. Further, an altered gut microbiota has also been linked to increasing one's risk for the development of neurological disorders. Specifically, metabolites produced by the gut microbiome can impact brain vascular function, such as by decreasing blood brain barrier permeability. Our objective was to examine the effects of the aging process on the gut and brain in Young and Old mice and how this collectively can affect neurological function. We hypothesized that brain function may be associated with the gut microbiome and over time, deleterious changes would be exhibited in both the brain and gut, effecting brain vascular, metabolic and cognitive functions. Young (5–6 mo.) and Old (18–20 mo.) male C57BL/6 mice were assigned to two groups (n = 19–20). Our multi-faceted approach included magnetic resonance imaging for measurement of cerebral blood flow (CBF), 16 S sequencing of the gut microbiome, brain metabolomics, blood brain barrier P-glycoprotein (P-gp)

transport, and behavior testing. All statistical analyses were completed using GraphPad Prism with significance reached if $p < 0.05$. Our preliminary results found that Old mice had significantly decreased CBF compared to the Young mice. Further, the Young and Old mice also had significantly different microbial diversity along with alterations in certain bacterial taxa. Moreover, the Old mice had altered brain metabolomics, decreased P-gp activity, and performed worse on behavior tests. We conclude that the aging process may indeed alter the gut microbiome and the brain. This shows promise that by modulating the gut microbiome, brain health might be optimized to help prevent neurological disorders. However, a greater understanding of the mechanisms connecting the gut and brain need to be elucidated.

PS06-067

Poster Viewing Session VI

Effects of aging and 17β-estradiol on glucose transporter (GLUT3) and membrane functions in female rat brain: A therapeutic potential drug for Alzheimer's disease

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Abstract

Recently, there has been a growing interest in the action and functions of the ovarian steroid hormone estradiol, particularly on whether they are neuroprotective for such age related disease and neurodegenerative conditions like stroke, Parkinson's disease and Alzheimer's disease. The objective of this study was to observe the changes in activities of mitochondrial enzymes (monoamine oxidase (MAO), Na+K+ ATPase and Ca^{2+}ATPase), membrane fluidity, DNA degradation, and glucose transporter 4 (GLUT3) expression in brains of female Wistar rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of 17β-estradiol (E2) (0.1 μg/gm body weight for one month).

Controls animals received an equal volume of vehicle. After 30 days of hormone treatment, experimental animals of all the groups were sacrificed and brains were isolated for further study.

The results obtained in the present work revealed that normal aging was associated with significant decrease in the activity of Na^+K^+ATPase, Ca^{2+}ATPase and membrane fluidity, GLUT3 levels in the brains of aging female rats, and an increase in DNA degradation and MAO activity. The present study showed that estradiol treatment significantly decreased DNA degradation, and MAO activity in brain of aging rats, and a reversal of Na^+K^+ATPase, Ca^{2+}ATPase and GLUT3 levels was achieved.

It can therefore be concluded that estradiol's beneficial effects seemed to arise from its antilipofuscin, antioxidant, antilipidperoxidative effects, implying an overall anti-aging action. The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders.

PS06-068

Poster Viewing Session VI

Correlation of gait and balance disturbances with cognitive impairment in patients with stable and unstable angina in Kyrgyzstan

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Abstract

Background: Falls and instability in walking are cause of morbidity in dementia, but there have been no studies of estimation the correlation between the level of cognitive impairment, level of anxiety and depression and balance and gait disturbances specific to this patient population in Kyrgyzstan.

Aim: To identify the correlation of gait and balance disturbances with cognitive impairment in patients with stable and unstable angina In Kyrgyzstan.

Materials and methods: We studied 73 patients with stable and unstable angina in the cardiology departments from 6 regions of Kyrgyzstan. To define cognitive impairment we used MMSE, Frontal Assessment Battery (FAB), Watson method of scoring clock drawings. For gait disturbances revealing we used Unterberger test, Tinneti balance scale. Emotional disturbances we estimated with Zung depression scale, State-Trait Anxiety Inventory (STAI).

Results: The mean age was 63.14 ± 8,9 years. Imbalance was identified in 42.47% patients, who were unstable in Romberg test and according to Tinetti scale 90.4% were at high and medium risk of falling. As a result of the MMSE 41.32% of patients had mild cognitive impairment (CI), 19% had moderate CI and in 11.57% we found dementia. Mild CI were observed in all age groups, the peak of which was accounted at 55 - 59 years. We used the Watson method of scoring clock drawings and found that 60% of patients did not manage performing. According to FAB 34,25% had dementia, and 45.21% moderate CI. Dementia was detected in 29 patients with unstable angina and in 15 patients with stable angina. Most of the patients had mild depression, moderate personal and high reactive anxiety.
Conclusion: In patients with stable and unstable angina we revealed significantly higher the risk of falls ($p = 0.001$) in the group with dementia, as well as in patients with high anxiety.

PS06-069

Poster Viewing Session VI

Effect of *Syzygium cumini* (jambolan) extract on spatial learning and memory of aging rats

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Abstract
Objective: Antioxidants help to inhibit oxidative damage caused by free radicals, a process that occurs in numerous brain related diseases. Jambolan extract have been extensively studied for a broad range of antioxidative activities. Therefore, the effects of the jambolan extract on learning and memory of aging rats were investigated.
Method: 18 months old male wistar rats ($n = 32$) were orally administered with vehicle, jambolan extract (JE) treated (500 mg/kg/day) or vitamin E (40 mg/kg; positive control) for 28 days. The learning and memory performance were monitored using Morris water maze (from day 21 to 30). All rats received four trails per day. On the last training day, all rats were tested in the probe trial.
Results: Vitamin E and the JE exhibit significant enhancement in memory, as shown by decrease in escape latency time, compared with day 1 ($p < 0.05$). The number of entries to the target quadrant of vitamin E and the JE group were higher, but not significantly different, than the control. Furthermore, Vitamin E and the JE (500 mg/kg) increased, but not significantly different, the time spent in the target quadrant during probe trial, indicating retention of spatial memory of the location of a previously placed platform in the target quadrant.
Conclusion: These findings indicate that JE has potential to improve age-related spatial memory impairment, which might be by virtue of its antioxidant activities. The exact mechanism of action remans unkown and need further extensive study.

PS06-070

Poster Viewing Session VI

Influence of sex on brain volume

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Abstract
It appears that there still remains to be no consensus on sex differences with regards to global brain gray matter volume and neurodegeneration. Understanding the influence of sex on global brain gray matter volume will ultimately provide a better understanding of any sex-biased neurodegeneration, and a closer understanding of any sex-biased cognitive and neurological diseases and conditions. Therefore, in this study we report on sex similarities and differences on global brain gray matter volume and the rate of decline in global brain gray matter volume across age, for both males and females, from 384 whole-brain magnetic resonance images.

PS06-071

Poster Viewing Session VI

Does the metabolic response to stroke explain the 'obesity paradox'?

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Abstract

Objectives: Obesity is a risk factor for stroke and worsens outcome in animal models. However, studies in patients often report better stroke recovery in the obese. Since inflammation is usually detrimental to stroke outcome, and obesity features a raised inflammatory profile, this finding has been called the 'obesity paradox'. A suggested mechanism for this protective effect is that obesity may improve tolerance to post-stroke weight loss (cachexia). Therefore, the aim of this study was assess whether there are metabolic differences in the responses of obese and lean mice to ischaemic stroke.

Methods: Focal cerebral ischaemia was induced by intraluminal filament occlusion of the middle cerebral artery in 16-to-20-week-old obese ob/ob and control (ob/-) mice. Ischaemic damage was assessed at 24 hours post-stroke, as were the metabolic and inflammatory responses to stroke. Lipid metabolism was assessed in the blood and in epididymal fat pads. Metabolomic analysis was performed on cardiac blood samples. Concentrations of inflammatory mediators were measured in epididymal fat, liver and plasma by cytometric bead array.

Results: Obese mice suffered increased ischaemic brain damage, and had clear differences in a range of metabolites after stroke. In particular, obese mice showed increased concentrations of plasma fatty acids and up regulation of lipolytic enzymes in adipose tissue. Stroke also resulted in an upregulation of inflammatory mediators in the adipose tissue of obese mice that was not seen in controls.

Conclusions: These results suggest stroke may differentially affect lipid metabolism in obese and lean mice, potentially related to an inflammatory response in the adipose tissue. This may have implications for understanding the 'obesity paradox', but may also mediate the increased ischaemic damage seen in obese mice since pathological concentrations of fatty acids may be pro-inflammatory.

PS06-072

Poster Viewing Session VI

Hemisphere-dependent metabolic and hemodynamic reorganization of the brain in elderly patients with ischemic stroke

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Abstract

Aim: Complex analysis of the metabolism, cerebral hemodynamic and their correlations in the elderly post-ischemic stroke patients with regard to hemispheric ischemia localization.

Subjects and Methods: Patients aged 60–75 years (n = 105) after an ischemic stroke in the MCA basin against a background of cerebral atherosclerosis and arterial hypertension at the period of their rehabilitation. Eighty-five individuals aged 60–77 years made a control group.

Duplex scanning of head and neck vessels was performed on the ultrasound device Sonoline Elegra (SIEMENS); magnetic-resonance spectroscopy of ^{1}H nuclei was done on the tomograph 1.5 T Magnetom Vision Plus (SIEMENS).

Results: At right-sided ischemic stroke (IS) the metabolic changes ((decrease of the NAA (N-acetylaspartate) and Cho (choline) contents)) were detected in the gray substance of the occipital area in the both, intact and damaged hemispheres, whereas at left-sided IS such changes were found only in the damaged hemisphere. In the patients with the focus of more than 60 cm3 the NAA content in the posterior and frontal lobes was statistically lower than in the patients with the focus of up to, 20 cm3. In all study patients the contents of main metabolites (NAA, creatine (Cr) and Cho) correlated with the volume blood flow velocity in the MCA of the intact and damaged hemispheres. At the right-sided IS localization, these correlations were more pronounced. In the control group the metabolite contents in the grey and white substance of the brain correlated with the volume blood velocity in the extracranial vessels of the carotid basin.

Conclusion: Evidence is presented about more marked metabolic changes in the grey and white matters of the brain and the dependence of main metabolites on hemodynamic indices in the intracranial sections of the MCA in the patients with right-sided localization of ischemic stroke.

PS06-073

Poster Viewing Session VI

Alzheimer's-induced alterations in cerebral energy metabolism measured with 2-photon fluorescence lifetime microscopy of intrinsic NADH

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Abstract

Objectives: Cerebral energy metabolism varies with aging and during the progression of neurodegenerative disorders such as Alzheimer's Disease. Detailed characterization of these metabolic alterations and its contribution to AD pathology requires measurement methods with high cellular resolution. Here, we utilize 2-photon (2P) lifetime microscopy to investigate changes of the intrinsic electron carrier reduced nicotinamide adenine dinucleotide (NADH) in an animal model of AD or induced by pharmacological manipulation. Fluorescence lifetime imaging enables resolution of different enzyme bound formulations (aka species) of NADH, providing insight into disease-related alterations in oxidative metabolism.

Methods: 2P imaging was performed through cranial windows in anesthetized Sprague Dawley rats or B6C3-Tg mice, the latter of which develop the primary morphological and cognitive hallmarks of AD. A custom-designed multimodal imaging system was utilized to measure various species of endogenous NADH. In rats, NADH was measured before and after addition of well-characterized pharmacological inhibitors of either glycolysis, the TCA/Kreb's cycle, the electron transport chain, or oxidative phosphorylation. In the AD-mouse model, NADH was measured around Aβ plaques and compared to age-matched, wildtype controls.

Results: Phasor analysis was implemented, providing a computationally-simple method to assess variations in fluorescence lifetime. Metabolic alterations are easily visualized on 2-dimensional phasor plots. Cerebral NADH lifetime measured around Aβ plaques or after pharmacalogical inhibition of the TCA cycle, electron transport chain, or oxidative phosphorylation appeared as separable phasor clusters in phasor space.

Conclusions: 2P fluorescence lifetime imaging extends the utility of NADH fluorescence measurement to indicate metabolic activity with greater specificity. AD progression induces distinct alterations in NADH lifetime that can easily be separated from wildtype controls. NADH lifetime measured around Amyloid-β plaques resembles that of brain tissue treated with inhibitors of the electron transport chain, indicative of Amyloidβ-related deficits in mitochondrial function.

PS06-074

Poster Viewing Session VI

Feasibility of opto-magnetic resonance spectroscopy at 9.4T

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Abstract

Introduction: Here, the possibility to couple optogenetics and MRS for a more specific evaluation of biochemical changes underlying optogenetics stimulation was explored.

Materials and Methods: 1H-MRS was performed at 9.4T (Bruker,Germany). Fisher rats (260 ± 50 g) underwent a craniotomy 4 mm lateral to the bregma at a 0.1 mm depth under isoflurane anesthesia in order to either inject an opsin (C1V1) 4 weeks prior to MRS or/and to insert an optical fiber (OF) into the S1FL on the day of MRS. Electrical (9 Hz, 1.2 mA) and optical stimulations1 (Blue or green lights,10 ms pulses, 9 Hz, 22 $mWmm^{-2}$) were performed (10 s ON-20 s OFF, 600 repetitions). Experiments were conducted under medetomidine. PRESS spectra were acquired in a VOI localized over the activated S1FL region encompassing the tip of OF. FASTMAP shimming was performed over the same VOI. LCmodel was used. Signal to noise ratio (SNR) was calculated as the ratio of the amplitudes of the NAA peak and of the signal between 0 and 0.5 ppm.

Results: In the presence of an OF, the water linewidth (lw) in a 18 µl VOI decreased down to 24.2 ± 5 Hz (n = 5) whereas naive rats (n = 3) demonstrated a lw of 19.4 ± 5 Hz. Proton spectra acquired in S1FL with and without OF had SNR levels of 60 and 55 respectively . Upon electrical stimulation, SNR levels were systematically higher than with blue or green laser lights without opsin. Proton spectra acquired during successive electrical and green laser stimulations in a C1V1 implanted rat (12 µl) showed SNRs of 27 and 38 respectively (Fig1a–1b) . The neurochemical profiles were obtained (Fig1.c)

Discussion and Conclusion: Feasibility of opto-MRS in rats depends highly on appropriate shimming and SNR. Although important optimization processes are needed, spectra were already of good quality ensuring that opto-MRS can be further developped.

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PS06-075

Poster Viewing Session VI

Improved cerebral energetics and ketone body metabolism in db/db mice

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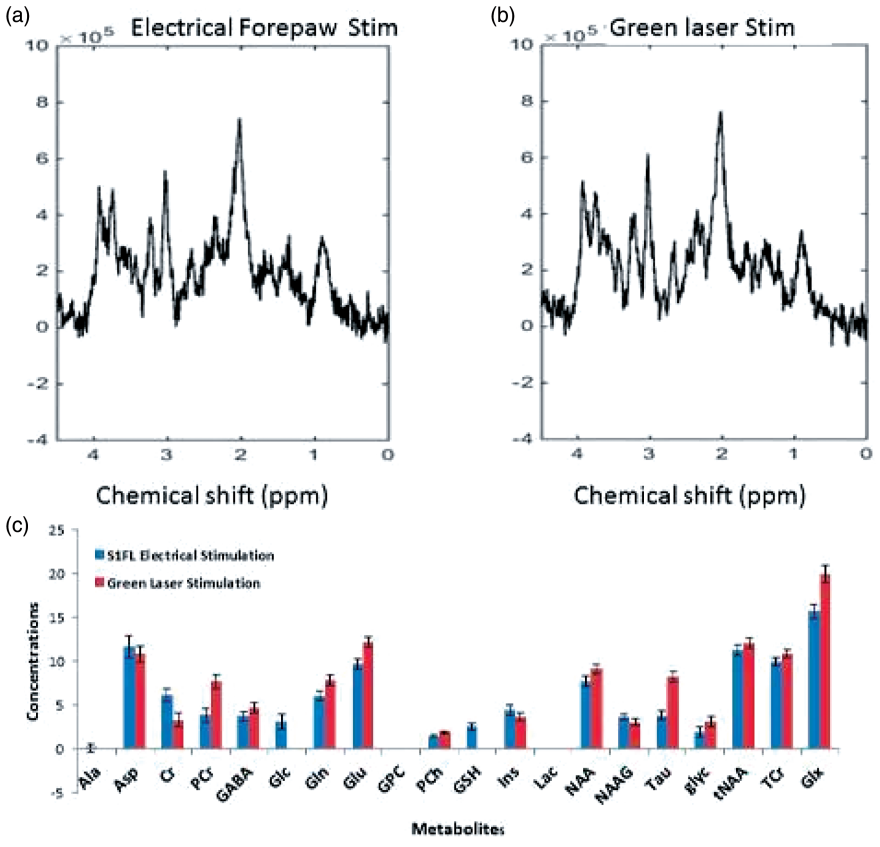
Abstract

Objectives: Type 2 diabetes mellitus (T2DM) is a risk factor for the development of Alzheimer's disease (AD). Hypometabolism of glucose has been observed in pre-symptomatic patients and animal models of AD, suggesting a fundamental pathological mechanism. The aim of this study is to elucidate cerebral metabolic consequences of T2DM to reveal accelerating factors of T2DM in AD pathology.



Methods: Db/db mice (16 weeks) were used as T2DM model. Cerebral cortical and hippocampal slices of db/db mice were incubated in media containing [U-13C]glucose or [U-13C]β-hydroxybutyrate. Oxygen consumption and ATP synthesis rate of isolated whole-brain mitochondria was assessed by SeaHorseXFe96 and on-line luciferase based assay, respectively.
Results: Decreased 13C enrichment from [U-13C]glucose metabolism were observed in key metabolites in extracts of both cerebral cortical and hippocampal slices of the db/db mouse. However the glucose hypometabolism was more prominent in the cerebral cortex. Incubations with the ketone body [U-13C]β-hydroxybutyrate showed increased 13C labeling in citrate, glutamate and glutamine in hippocampal slices of db/db mice. These changes were absent in the cerebral cortex. Isolated whole-brain mitochondria from db/db mice, surprisingly displayed augmented oxygen consumption when stimulated with ADP, when pyruvate and malate were provided as substrates. This finding was supported by a significantly increased ATP production from isolated brain mitochondria of the db/db mice.
Conclusion: Cerebral metabolism and energetics are affected in the db/db mouse. Hypometabolism of glucose is evident in the cerebral cortex and hippocampus. However, the hippocampus of db/db mice, exhibits augmented ketone body metabolism. Mitochondria isolated from the db/db brain showed a significant increase in respiration and ATP synthesis. The results suggest that the hypometabolism of glucose is the major deleterious effect of T2DM on brain energy metabolism. However, the increased ketone body utilization and augmented mitochondrial efficiency suggests compensatory mechanisms, possibly related to the glucose hypometabolism, in the diabetic brain.

PS06-076

Poster Viewing Session VI

Activity-induced cellular lactate changes in the brain

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Abstract
Lactate elevations in the brain upon activation have been described several decades ago and were primarily taken as evidence for a mismatch between glycolysis and respiration. However, to completely establish the full biological significance of activity-induced cerebral lactate elevations methods that resolve events on a cellular level are necessary. The advent of the new genetically encoded lactate biosensor Laconic, which can be expressed in vivo in a cell-specific manner using respective promoters and adeno-associated viruses, allows the observation of lactate levels in single cells. In combination with two-photon laser scanning microsopy we examined lactate concentrations in astrocytes and neurons in the cerebral cortex of anesthetized mice upon increased activity. With the onset of intracortical microelectrode stimulation transient lactate increases in neurons and astrocytes were observed. After cessation of the 1-min stimulation period lactate levels did not return to baseline immediately. In experiments, where we applied the lactate dehydrogenase blocker oxamate the stimulation-induced increases in lactate concentration diminished significantly in both astrocytes and neurons by 50%. In a second cohort of mice, we investigated the dynamics of the upstream substrate glucose during the same stimulation protocol using the genetically encoded glucose sensor FLII^{12}Pglu600μΔ6. Interestingly, glucose levels exhibited stimulation-induced decreases only in astrocytes whereas neuronal levels remained stable.
Previously observed cerebral lactate elevations are now for the first time assigned to astrocytes and neurons. Based on our results, lactate seems mainly be derived from cerebral metabolism. However, future experiments should be designed to answer the questions about the exact cellular origin of the lactate increases.

PS06-077

Poster Viewing Session VI

Masticatory muscle metabolic dysfunction and neuronal excitability induced by unpredictable chronic stress in rats

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Abstract
The patient has dental losses reveals an occlusal collapse influencing stomatognathic functions. Although stress be involved in the pathogenesis of chronic diseases and enable the masticatory muscle pain in patients, it is still not understood the mechanism by which muscle adapts to psychological and physiological stress in orofacial muscle dysfunction and which neural pathways are involved in this process. This study investigates the effect of chronic stress associated with unilateral extraction on the medial pterygoid muscle of rats and neural activity of brain areas and the influence of pre -treatment with diazepam. Wistar rats were divided in with or without stress and diazepam administration or your vehicle. The rats were euthanized to obtain the medial pterygoid muscle and brain and immunohistochemical analysis for reactive oxygen species (ROS) and c-fos and fos B were performed. The results indicate an increase of ROS in groups subjected to stress and extraction, together or separately (Two Way ANOVA, Student-Newman-Keuls, $P < 0.01$). The group subjected to stress and treated with vehicle (63.8 ± 1.5) was different from the group treated with benzodiazepines (51.6 ± 2.46) (Student-Newman-Keuls, $P < 0.05$), where the treatment for 10 days, was effective in reducing ROS in the muscles of animals subjected to chronic stress. Quantitative analysis indicates increased neuronal activity in amygdala, hippocampus and trigeminal nucleus after tooth extraction and stress protocol. Diazepam reduced neuronal labeling during the stress in the limbic system, and in trigeminal nuclei. This study indicates that stress were able to increase ROS in the medial pterygoid muscle and FOS in brain regions and shows reversion of this situation through benzodiazepine therapy. Use of this treatment protocol can be effective in the attenuation of pathological situation by stressors in masticatory muscles.

PS06-078

Poster Viewing Session VI

Signaling in brain via cAMP is decreased in unmedicated depressed patients and normalizes with drug treatment

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Abstract
Background: One theory of depression posits that the disorder is caused by low cAMP signaling and that chronic, but not acute, administration of antidepressants upregulates cAMP signaling. We examined this theory in patients with major depressive disorder (MDD) using the PET radioligand [11C](R)-rolipram, a reversible inhibitor of phosphodiesterase-4 (PDE4). Because of a biochemical feedback mechanism, the binding of rolipram to PDE4 provides a measure of the activity of this enzyme.
Methods: By quantifying radioligand brain uptake relative to that in arterial blood, we measured PDE4 binding in 44 unmedicated patients with MDD and 35 healthy controls, and performed a second scan in 23 patients after two months treatment with SSRI (sertraline or citalopram).
Results: Consistent with our prior finding in 28 unmedicated patients, the current enlarged sample in 44 patients found ~18% decreased PDE4 binding in all regions of brain compared to control subjects ($p = 0.001$). As to the effect of SSRI (which has not previously been reported), two months treatment in 23 patients increased PDE4 binding ~11% ($p = 0.000$) in all brain regions, but increased PDE4 binding was not correlated with symptom improvement.
Conclusions: Consistent with the cAMP theory of depression, PDE4 was decreased in unmedicated MDD patients and increased after two months treatment with SSRI. The lack of correlation of increased PDE4 binding with symptom improvement could reflect the heterogeneity of the disease and/or the heterogeneity of the target, as PDE4 has four subtypes, only one of which may be involved in antidepressant response.

PS06-079

Poster Viewing Session VI

The effects of progesterone administration in patients with diffuse axonal injury

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Abstract
Background and Objectives: To permit appropriate targeted therapy, the present clinical study was aimed to investigate the effects of progesterone on the outcome and the serum markers of injury, oxidant activity and inflammation in diffuse axonal injury (DAI). And, there has been interest in the importance of serum parameters as predictors of outcome in traumatic brain injury.
Patients and Methods: Thirty-two male DAI patients (18–60 years of age, a Glasgow coma scale of 12 or less, and admitted within 4 hours after injury) who were randomized for a controlled phase II trial of progesterone. The authors analyzed neurologic outcome [Extended Glasgow Outcome Scale (GOS-E) and functional independence measure (FIM)], the markers of inflammation [interleukin-1β (IL-1β), IL-6, intercellular adhesion molecule-1 (ICAM-1), transforming growth factor-β1 (TGF-β1)], injury [brain proteins of S-100B and oxidant activity [malondialdehyde (MDA)] in these patients.
Results: Higher GOS-E and FIM scores were observed in progesterone group at the six-month follow-up ($P < 0.05$ and $P < 0.01$, respectively). Mean while, a reduction in the serum levels of IL-1β, ICAM-1, MDA and S-100B was noticed in progesterone group 24 after injury ($P < 0.05$, 0.05, 0.001 and 0.05, respectively), and there was an increase in serum levels of IL-6 and TGF-β1($P < 0.01$ and $P < 0.05$, respectively). Also, lower levels of MDA and S-100B, and higher levels of TGF-β1 were observed in progesterone group six days after injury ($P < 0.05$).
Conclusions: In summary, progesterone administration may improve the outcome in DAI patients probably through modulation in the levels of cytokines, and reduction in the injury and oxidant activity.

PS06-080

Poster Viewing Session VI

Temozolomide arrests glioma growth and normalizes intratumoral extracellular pH

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Abstract
An acidic extracellular pH (pH_e) of gliomas promotes tumor growth and builds resistance to therapy. Given evidence that acidic pH_e beyond the tumor core indicates infiltration, we hypothesized that imaging the intratumoral pH_e in relation to the peritumoral pH_e can provide a novel readout of the changing tumor microenvironment with therapy. We used Biosensor Imaging of Redundant Deviation in Shifts (BIRDS), which utilizes shifts of non-exchangeable protons from macrocyclic chelates (e.g., 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate) or $DOTP^{8-}$) complexed with paramagnetic thulium (Tm^{3+}), to generate pH_e maps in rat brains bearing U251 tumors. Upon $TmDOTP^{5-}$ infusion, MRI identified tumor boundary and BIRDS imaged the pH_e gradient between intratumoral and peritumoral regions (delta pH_e). Approximately two weeks after implantation of U251 glioma cells, animals were either treated with temozolomide (50 mg/kg) or were left untreated. Reduced proliferation (by Ki-67; $p < 0.03$) and induction of apoptosis (by cleaved Caspase-3; $p < 0.001$) were observed in treated rats compared to untreated rats. Tumor growth, measured at approximately three weeks (after glioma implantation), was inhibited in treated rats compared to untreated rats ($p < 0.03$). The delta pH_e was significantly higher in untreated rats compared to treated rats ($p < 0.002$). These results suggest that temozolomide therapy, which induces apoptosis, hinders tumor growth and proliferation while at the same time normalizes intratumoral pH_e. In summary, BIRDS can be used to map the delta pH_e in glioma treatments (e.g., pH_e and pO_2 targeted drugs) to provide a metabolic readout of the tumor microenvironment.

PS06-081

Poster Viewing Session VI

The structural atrophy is associated with CSF neurofilament light chain in a transgenic rat model of Alzheimer's disease

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Abstract

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disease that has multiple pathophysiological characteristics such as amyloidosis, neurofibrillary tangles, and atrophy. By using in vivo deformation based morphometry (DBM), even small structural changes can be visualized. Furthermore, cerebrospinal fluid (CSF) neurofilament light (NfL) is thought to reflect axonal and neuronal damage. However, the association between the imaging and fluid biomarker needs further investigations. Here, we utilized McGill-R-Thy1-APP transgenic rat (Tg) model1, which accumulates amyloid pathology without NFTs or cell depletion. We aimed to investigate the link between CSF-NfL and DBM. We hypothesized that the CSF-NfL level is associated with the regional atrophy in the Tg.

Methods: A total of 28 (11 WT, 17 Tg) rats underwent CSF collection (10–17 months). CSF-NfL concentration was measured using a novel ultrasensitive Single molecule array (Simoa) method2. Also, the structural MRI following 8-angles FISP with a TR/TE = 2.5/5.0 ms for 52 min was performed at 21 months. DBM was generated from the linear and nonlinear transformations onto the sample average template3. We have selected only the atrophic regions in Tg compared to WT and employed in the multiple regression model: DBM ~ NFL10–17 mo + Sex. The statistical analysis of voxel-wise parametric map was performed using VoxelStats4.

Results: The CSF-NfL level is significantly associated with the structural atrophy in both grey and white matter in corpus callosum, hippocampus, entorhinal cortex, nucleus accumbens, thalamus, striatum, and midbrain regions in Tg.

Conclusion: Our results revealed that the CSF-NfL level is associated with the structural atrophy in medial basal cortex and subcortical regions.

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2. *Recent Advances and the Future Generation of Neuroinformatics Infrastructure* (2015): 227.
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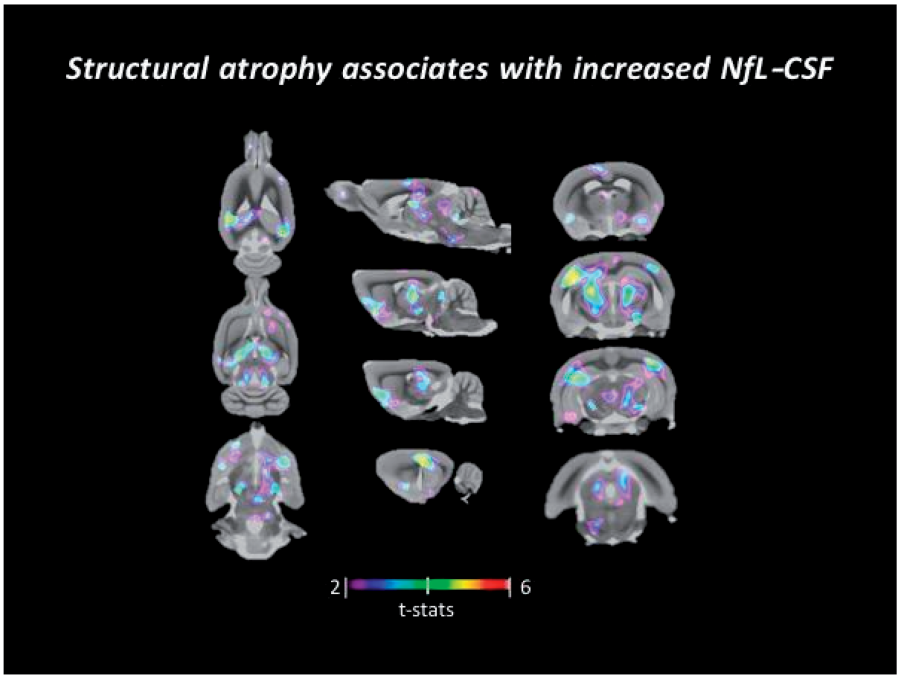

PS06-082

Poster Viewing Session VI

T-cell stimulation in human stroke

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Abstract

Objective: Antigen presenting cells have been described in the CNS after stroke, and neural antigens were detected in draining lymphoid tissues of stroke patients. The aim of this study was to investigate cytokine production and brain antigen-dependent responses in lymphocytes of stroke patients.

Methods: PBMC from age-matched control donors (n = 10) and stroke patients (n = 13) at day 1, 5 and 90 post-ischemia were cultured with neural peptides for 24 h and functional activation of T cells was assessed by cytokine production using flow cytometry. The peptides were: a) antigenic peptides from human microtubule-associated protein 2 and N-methyl-D-aspartate receptor subunit 2A selected in silico based on immunogenicity and ability to bind to HLA, and b) pools of peptides of human myelin basic protein and myelin oligodendrocyte glycoprotein. Polyclonal T cell reactivity was explored by stimulation with PMA/Ionomycin. Functional recovery of the patients was assessed at 3 months using the modified Rankin scale.

Results: Compared with controls, CD3 lymphocytes of stroke patients showed a non-significant increase in the basal production of IFN-g, TNF-α, IL10 and IL-17 at day 1 post-stroke. IFN-g and TNF-α significantly decreased at day 5 vs. day 1. On the contrary, IL-4 significantly increased over time. After PMA/Ionomycin stimulation, lymphocytes were more activated at day 1 vs. day 5 after stroke. Net cytokine production after stimulation with neuroantigens was low. In exploratory analyses, reactive lymphocyte TNF-α production was significantly lower in the subgroup of patients with good outcome at follow-up (n = 7).

Conclusions: Lymphocyte cytokine production was down-modulated 5 days post-stroke with the exception of increased production of the anti-inflammatory and M2-polarising IL-4. Overall, the lymphocyte response to neural antigens was poor. Stroke-induced immunodepression may prevent developing deleterious antigen-specific T-cell responses. Further studies with larger series of

patients and additional stimulation strategies are needed to confirm these results.
Supported by FIS (PI12/01437)

PS06-083

Poster Viewing Session VI

Brain-targeting adaptive immune responses in extracorporeal membrane oxygenation-treated patients

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Abstract
Objectives: Extracorporeal Membrane Oxygenation (ECMO) is a life saving technique that provides cardiac and respiratory support to persons with heart/lung dysfunction. Neurological injury contributes to morbidity and mortality in patients undergoing ECMO and induce peripheral inflammation. We hypothesized that ECMO induces CNS-directed autoreactivity which contributes to neurological injury.
Methods: Using a single center prospective observational study, we of ECMO patients 0–18 years old, we isolated PBMC to quantify innate and adaptive immune cells by flow cytometry A recall-response assay detected autoreactive responses to myelin basic peptide (MBP), proteolipid lipoprotein (PLP), myelin oligodendrocyte glycoprotein (MOG), NMDA receptor (GluN2A), and microtubule associated protein (MAP2), with fluorescent antibodies targeting CD4, CD19, CD8, CD25 proteins.
Results: ECMO patient's PBMC were sampled at day 1 ($n = 12$), day 3 ($n = 11$) and day 7 ($n = 4$) with non-ECMO sick patient controls ($n = 5$). Six ECMO patients presented with brain injuries determined by MRI. When comparing between time points and disease controls, we did not see a significant difference in the general leukocyte populations (CD45+), natural killer cells (CD161+), monocyte (CD14+), T-cells (CD3+), B-cells (CD19+) and cytotoxic cells (CD8+), thoughCD4+ T cell responses were elevated on day 3 in patients with brain injury. We observed a significant increase in activated macrophages (day 3 vs. day 7; $p < 0.05$, CD14+CD11b+). Recall response to CNS autoreactivity showed myelin (MOG and PLP) responses at day 1,3 and 7, from patients with acquired brain lesions or subdural hemorrhages.
Conclusion: ECMO patients with brain injury exhibit an increase in activated macrophages and a trend toward increased CD4 T-cells, which suggests altered immune trafficking. In conjunction with the detection CD4 myelin-specific response in ECMO treated patients, it could be proposed that ECMO treatment allows for adaptive activation directed toward CNS antigens, though future analysis will determine long-lasting cognitive deficits in these patients at a one-year follow-up.

PS06-084

Poster Viewing Session VI

Impairment of vasopressin secretion in sepsis survivor rats

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Abstract
Objectives: Previous studies have shown impairment in the osmoregulatory and hypotensive/hypovolemic control of arginine vasopressin (AVP) secretion during the sepsis. Here we investigated the persistent neuroendocrine dysfunction in sepsis survivor rats.
Methods: Wistar rats were submitted to sepsis by cecal ligation and puncture (CLP) and compared with naive (control group). In a first set of experiments, the rats were submitted to CLP and the survival rate was monitored for 10 days. In a second set of experiments, sepsis survivor animals were intraperitoneally injected with hypertonic saline (2 M) or polyethylene glycol (2 mg/ml).

After 30 min or 90 min, respectively, blood was collected for determination of plasma AVP and oxytocin (OT) levels. In another group, hypertonic saline were administrated and the water, food intake and urinary volume were measured for 240 min.

Results: Osmotic challenge increased AVP and OT secretion in both groups but AVP secretion was attenuated in the survivor animals. Hypovolemic stimulus also increased both hormones, but differently from osmotic stimulus, there was no difference in sepsis survivors animals. Additionally, we did not observed alterations in water, food intake, and urinary volume following hypertonic saline injection in the sepsis survivor group.

Conclusions: Our data provide first experimental demonstration of persistent impairment AVP secretion followed by osmotic challenge in sepsis survival animals, highlighting the importance of this experimental model to study the consequences of surviving sepsis.

References:

Siami S, Polito A, Porcher R, Hissem T, Blanchard A, Boucly C, et al. Thirst perception and osmoregulation of vasopressin secretion are altered during recovery from septic shock. PLoS One. 2013;8(11):e80190.

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PS06-085

Poster Viewing Session VI

Defining mitochondrial biomarkers and function using magnetic resonance spectroscopy at 14.1 Tesla in a mouse model of mood disorders

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Abstract

In vivo magnetic resonance imaging(MRI) and spectroscopy(MRS) are two non-invasive techniques of choice for investigating and monitoring brain metabolic and biological changes related to mitochondrial function and health. Mitochondria have been associated with many brain disorders and, among them, mood disorders (Chaturvedi & Flint Beal, 2013). Defining and understanding mitochondrial MRI/MRS biomarkers related to mood disorders could be an important contribution for a better endophenotypic characterization of these psychiatric illnesses.

In this study we have investigated the MRI/MRS profile of a new mouse model of mood disorders lacking an important brain plasticity gene, *Crtc1* (CREB-regulated transcriptional coactivator 1). (Breuillaud *et al.,* 2009; Breuillaud *et al.,* 2012).

Metabolic and volumetric profile alterations were determined with T_2-weighted MRI together with ^{1}H-MRS of prefrontal cortex(PFC) and dorsal hippocampus(HDors). Results indicated an age-dependent alteration of glutamate and GABA levels in *Crtc1* KO mice PFC together with a constant reduction in phosphocreatine(PCr) energy metabolites in the dorsal hippocampus (PFC: Glu(-12 ± 3%), GABA(-26 ± 11%); HDors: PCr(-20 ± 8%)). qPCR experiments revealed no changes in electron transport chain(ETC) gene expression but increased creatine kinase(CKMt and CKB) levels in the dentate gyrus of KO mice, confirming neuroenergetic deficiency in dorsal hippocampus. Mitochondria quantification using mtDNA copy number revealed a specific reduction of mitochondrial mass in the dentate gyrus, which could explain the observed energetic dysfunction. Finally, preliminary 1H[13C]-MRS results upon infusion of [U-13C]glucose suggested metabolic differences in the dorsal hippocampus, where enrichment curves indicate a reduced glucose uptake together with an increased TCA cycle rate in KO animals. Together, these results suggest that CRTC1 might be an essential regulator of brain energy metabolism in the mouse dorsal hippocampus. Further investigations will aim at clarifying the mitochondrial failure of these mice and monitor its evolution with its associated MRI/MRS profile.

PS06-086

Poster Viewing Session VI

Activation-induced lactate changes in the human brain by J-edited 1H-MRS

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Abstract
Objectives: While functional MRI (fMRI) is used to localize regions of activation, functional MRS (fMRS) provides metabolic response to functional activation. fMRS, using short echo-time (TE) non-edited 1H-MRS protocols, has detected a robust lactate increase in sensory-induced activations. Given that short TE non-edited 1H-MRS is susceptible to functional hyperemic effects, we posited if long TE lactate J-edited 1H-MRS can reliably detect such metabolic changes.
Methods: Nine healthy volunteers (right-handed) participated in the single day experiment that consisted of single fMRI run and single fMRS run. Six participants came for a second fMRS run on another day, which resulted in total 15 fMRS runs. The fMRI run consisted of five 97 s regulation blocks that were interleaved with five 97 s baseline blocks (16.2 min). The fMRS run consisted of three 5 min finger-tapping blocks interleaved with three 5 min fixation blocks (30 min). Subjects were asked to perform the visually-cued finger tapping at a rate of 3 Hz. Numbers from 1 to 4 for each of four alternated fingers were indicated. Left motor cortex (MC) was identified using statistical parametric mapping (SPM12). Single voxel for J-editing acquisition protocol was placed around the identified area.
Results: We found significantly higher normalized lactate level during tapping as compared to fixation conditions ($t = 2.4$, $p = .03$, $df = 14$, paired two-tailed t-test) and no difference between the corresponding NAA levels ($p > .5$).
Conclusions: Our J-edited fMRS results at relatively low magnetic field (4T) showed the physiological modulation of the MC lactate level estimates during standard finger-tapping experiment. In summary these results confirm that the previously detected lactate changes are probably devoid of functional hyperemic effects.

PS06-087

Poster Viewing Session VI

Neurovascular niche: effects of Angiotensin II

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Abstract
In adult brain, neuronal stem cells (NSC) are located in the neurovascular niche therefore, vascular cells play an important role in creating a healthy environment for adult neurogenesis. Several laboratories including ours have demonstrated that Angiotensin II (AngII)-induced hypertension affects numerous vascular functions by causing oxidative stress, vascular inflammation and endothelial dysfunction. Further, the available evidence suggests that blood-brain barrier (BBB) deregulation is an indirect consequence of cerebrovascular inflammation. Of these, AngII effect on BBB dysfunction has been reported, and is implicated in the pathogenesis of stroke, small vessel disease, vascular dementia and Alzheimer's disease. In this regard, miRNAs emerged as key players in the development of vascular and neurodegenerative diseases. In here, we studied the effects of AngII on adult NSC and brain endothelial cell (BEC) miRNAs regulation and their interaction focusing on a specific miRNAs that were selected due to their role in other vascular systems. We used primary mouse NSC and BEC culture model to study the effects of AngII on miR-15, -34a, -155 and -let7 expression using qPCR. AngII 100 nm induced expression of miR-15 in both neurospheres and BEC by 2 and 2.5-fold respectively after one hr. miR-34 a expression was induced by 3.5-fold in neurospheres and by 3-fold in BEC in 24-hr. Mir-155 expression was elevated in neurospheres by 2-fold and in BEC by 3-fold after 45-min. miR-let7 was increased in neurospheres and BEC by 4-fold in 15 min, and remained induced in EC for one hr. These miRNAs expression changes were accompanied by increased protein expression of C/EBPb, pCREB, and p38MAPK. However, Ets1 expression was downregulated in both neurospheres and BEC. These results indicate that AngII has major effects on NSC and BEC through deregulating miRNAs and protein levels involved in this phenomenon. Further studies are needed to explore the mechanisms involved.

PS06-089

Poster Viewing Session VI

Interleukin-6 levels in cerebrospinal fluid and plasma in patients with severe spontaneous subarachnoid hemorrhage

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Abstract

There is substantial evidence that inflammatory processes play a key role in the pathophysiology of spontaneous subarachnoid hemorrhage (SAH) [1]. This study evaluated the inflammatory response in the acute phase after SAH and researched whether different temporal patterns of systemic and intrathecal inflammation could be identified. The intensity of the inflammatory response was also assessed in different clinical subgroups.

Forty four patients with severe SAH were included (median WFNS score = 4, median Fisher scale = 4 on admission). Cerebrospinal fluid (CSF) and blood samples were collected at days 1, 4 and 10 after ictus. Interleukin-6 (IL-6) was analysed by a routine monoclonal antibody-based method (Roche CobasE 602; reference interval for plasma < 7.0 ng/L; for CSF not available). Median IL-6 values for each day were calculated. Day 4 IL-6 values were considered indicator of the intensity of inflammatory response and compared in dichotomised subgroups (age, sex, WFNS score, Fisher scale, vasospasm, CNS infection, systemic infection).

Median IL-6 concentrations for day 1, 4 and 10 in CSF were 876.5, 3361 and 1567 ng/L while in plasma 26, 27.5 and 15.9 ng/L, respectively. The most prominent difference in day 4 IL-6 concentrations in CSF could be seen in WFNS score-based subgroups (1–3 vs 4–5 = 1158.5 vs 5538 ng/L, $p = 0.056$). No significant differences could be observed in any other subgroup. Patients with systemic infection had significantly higher IL-6 concentrations in plasma (31 vs 16.05 ng/L, $p = 0.028$).

A distinctly different inflammatory pattern both regarding its development over time and its intensity was seen intrathecally compared to the systemic circulation, indicating endogenous cytokine production within the CNS. Significant difference in the intensity of the inflammatory response in plasma was seen in cases of systemic infection but in no other subgroup, likely due to the homogeneity of the patient cohort.

Reference:

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PS06-090

Poster Viewing Session VI

Metabolic changes in developing brain due to chronic liver disease: an *in vivo* longitudinal and multiparametric study using ^{31}P-MRS (magnetic resonance spectroscopy), ^{31}P-Magnetization transfer and ^{1}H-MRS

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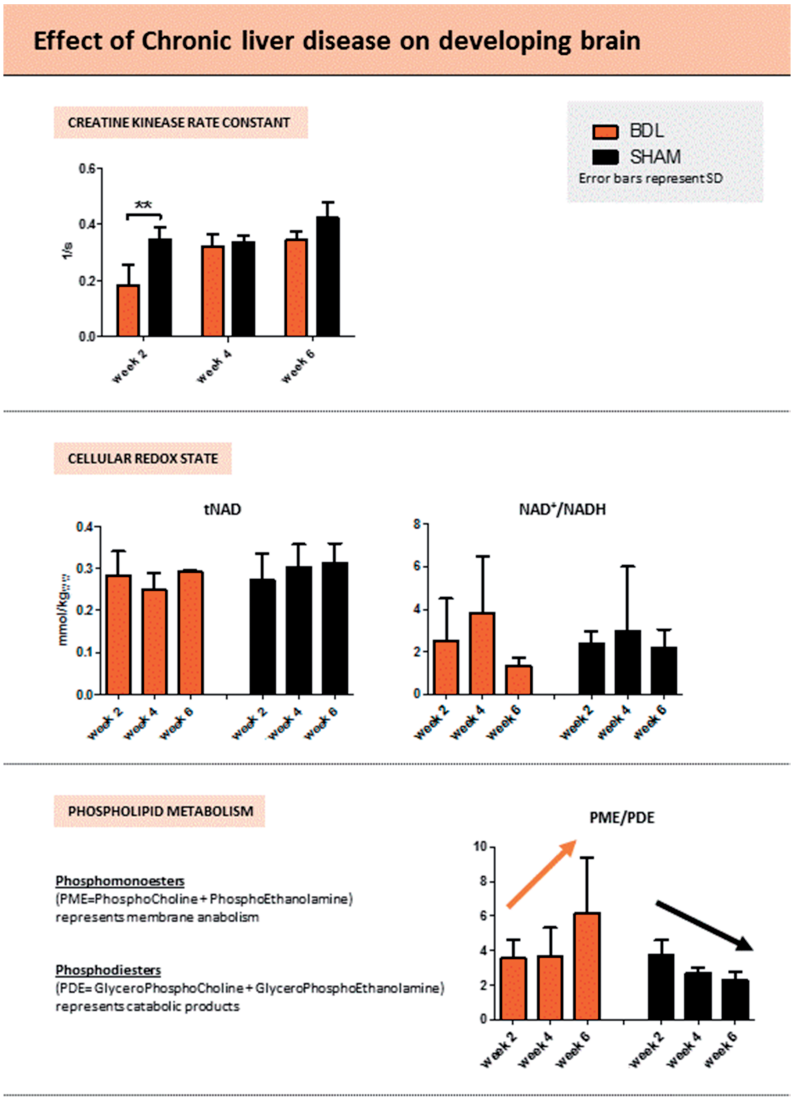
Abstract

Objectives: Chronic liver disease(CLD) is a serious condition leading to neurocognitive deficits referred to as chronic hepatic encephalopathy(CHE) with incompletely understood molecular basis.

Our aim was to perform a multi-parametric *in vivo* and longitudinal study to assess brain metabolism in developing brain during CLD by combining static and kinetic high field 31P-MRS and 1H-MRS.

Methods: Four rats (Wistar/male) were bile duct ligated (BDL) 21-days post-natal. Four rats underwent sham surgery at matched age. MRS brain studies were performed at week2/4/6 post-BDL (with blood sampling) on a 9.4T-system in VOI = 5×9×9mm3. Creatine kinase(CK) rate constant($k_{ATP\rightarrow PCr}$) was estimated using a 31P-saturation transfer. Subsequently, a static 31P-MRS and 1H-MRS were performed.

Results: BDL-rats showed typical signs of CLD (increase of plasma bilirubin(14 mg/dl),increase of brain glutamine(+320%), decrease of brain myo-Inositol(–37%)). $k_{ATP\rightarrow PCr}$ was significantly decreased at week2. Later



recovery of $k_{ATP \rightarrow PCr}$ could be related to compensatory mechanisms.

tNAD(NAD^++NADH) pool did not change, however, the observed fluctuating ratio of NAD^+/NADH indicates instable cellular redox state, in agreement with ascorbate and GSH decrease and lactate increase measured using 1H-MRS.

In addition, BDLs had markedly altered phospholipid metabolism (decreasing PDE, leading to increased PME/PDE ratio).

These preliminary results have to be validated on larger group of animals.

Conclusion: 31P-MRS revealed an altered phospholipid metabolism and fluctuations in mitochondrial function, associated with CLD-induced HE in developing brain.

Oxidative stress may be responsible for varying cellular redox state as well as alterations in $k_{ATP \rightarrow PCr}$. CK is susceptible to oxidative stress, resulting either in its impaired functions or compensatory up-regulation of its gene expression.

Phospholipid metabolism could be also altered due to increased oxidative stress by membrane lipid peroxidation.

These changes in phospholipid metabolism seem to be characteristic for developing brain during CLD since it was not observed in adults with CLD.

PS06-091

Poster Viewing Session VI

Evaluation of hemodynamic impairments in unilateral high-grade carotid artery stenosis patients and healthy age-matched participants

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Abstract

Objectives: Internal carotid-artery stenosis (ICAS) is a major public health issue, as it accounts for approximately 20% of all strokes1. However, related complex hemodynamic impairments are not well understood2. We therefore propose a multimodal MRI-protocol. The major aims were to evaluate its reliability and to investigate physiological changes.

Methods: In the ongoing clinical study, 52 subjects (29 healthy controls: 70.3 ± 4.7 y, 13 males; 23 patients with asymptomatic unilateral ICAS, NASCET>70%: 70.5 ± 6.8 y, 15 males) underwent MRI on a Philips 3T-Ingenia.

We propose a combination of three different MR-based methods, accounting for cerebrovascular reactivity (CVR) by breathhold-fMRI (voxelsize 3×3×3mm3, 38 slices, TE/TR = 30 ms/1200ms, acq.time = 5:48 min), CBF by pCASL (3D-readout, voxelsize 2.7×2.8×6mm3, 16 slices, TE/TR = 7.4 ms/4403ms, label duration = 1800 ms, PLD = 2000 ms, acq.time 5:43 min) and relative oxygen extraction fraction (rOEF) by a multi-parametric quantitative-BOLD approach3 (voxelsize 2×2×3mm3, 30 slices). For each participant, individual masks of watershed areas were defined for both hemispheres in grey-matter and mean values of all three modalities were compared.

Results: In healthy participants, our results show no significant lateralization of all three modalities on a group level. For ICAS-patients, regionally reduced CVR ($p = 0.003$) as well as hypo-perfusion ($p < 0.001$) were found ipsilateral to the stenosis (figure). In accordance with the literature, we did not find ICAS-induced changes in oxygen extraction on a group level ($p = 0.310$).4 Even though focal rOEF increases could be suspected in single patients.

Conclusions: The presented preliminary results thus imply successful application of our multimodal-MRI approach and are highly promising with respect to gaining a deeper insight into ICAS-related physiological changes. Further investigations of the relations between the parameters are currently in progress.

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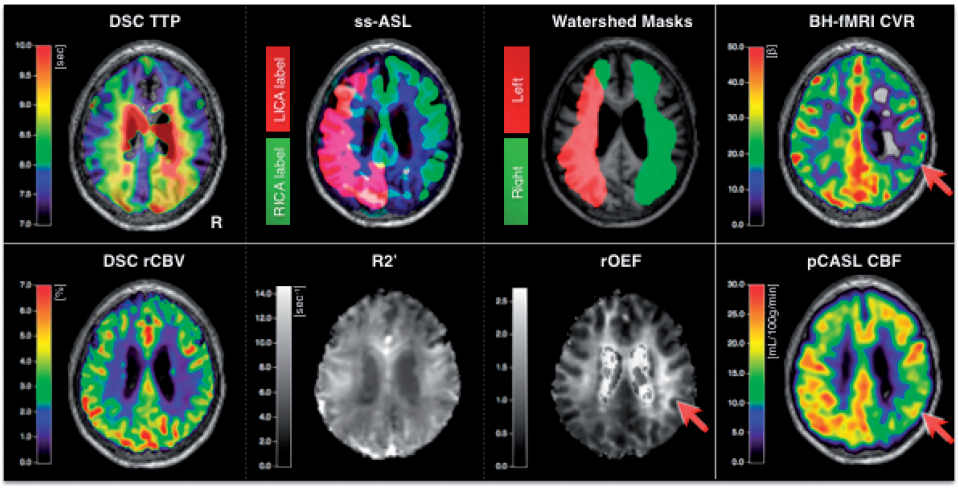

PS06-092

Poster Viewing Session VI

TOR drives fibrillar amyloid-β accumulation and cerebrovascular dysfunction in the Tg2576 model of Alzheimer's disease

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Abstract
Cerebral amyloid angiopathy (CAA), characterized by neurovascular fibrillar amyloid β (Aβ) accumulation, is present in up to 90% of patients with Alzheimer's disease (AD). To determine the role of mTOR in the pathogenesis of CAA and associated cerebrovascular dysfunction, we used intravital two-photon microscopy though an intact skull to measure fibrillar Aβ lesions as well as vascular reactivity in control- and rapamycin-treated 18–19 month-old Tg2576 mice. Further postmortem histological analysis and immunofluorescent confocal microscopy was performed to assess the consequences of fibrillar Aβ vascular deposition on BBB integrity and cerebrovascular damage in the same mice that were used for in vivo optical microscopy.
The present studies demonstrate that mTOR attenuation by rapamycin reduces the quantity and density of brain vascular Aβ lesions, preserves blood brain barrier (BBB) integrity and maintains brain vascular reactivity even in the presence of high vascular fibrillar Aβ load in Tg2576 mice, a mouse model of AD that recapitulates AD-associated CAA. Further, the maintenance of BBB integrity by chronic mTOR attenuation in Tg2576 transgenic mice reduced the number of cerebral microhemorrhages associated with CAA-like lesions. Importantly, the restoration of cerebrovascular integrity and reactivity by mTOR attenuation was associated with mitigated contextual memory deficits in the same Tg2576 mice that were used for in vivo optical imaging. These data, together with our recent published studies in the APP(J20) AD model, indicate that mTOR contributes to the development of CAA-like lesions, BBB breakdown, Aβ-induced cerebrovascular dysfunction, and cognitive deterioration in various independent mouse models of AD. Thus, mTOR inhibitors such as rapamycin, an FDA-approved drug, may have promise to treat AD and potentially other dementias that have vascular dysfunction as a common etiology.

PS06-093

Poster Viewing Session VI

One-sided hypoperfusion is associated with contralateral attention deficits in asymptomatic high-grade carotid-stenosis patients

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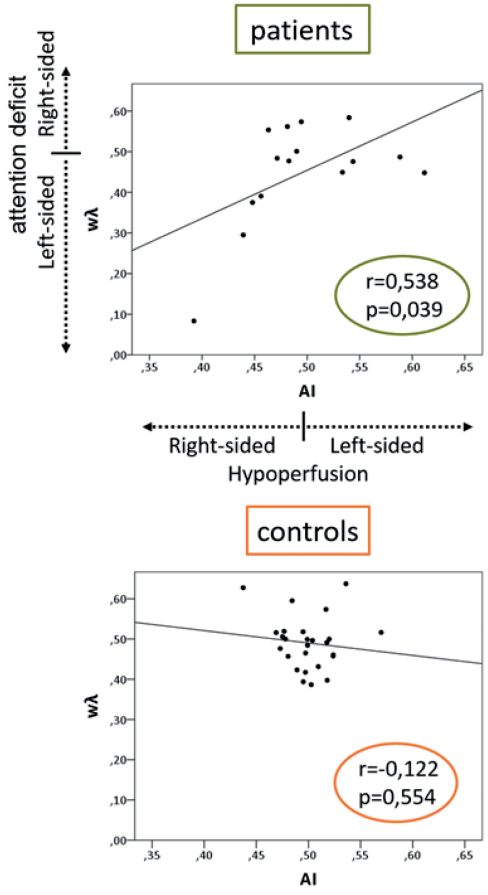
Abstract
Objectives: Patients with clinically asymptomatic, high-grade internal carotid artery stenosis (ICAS) often show cognitive impairments, such as memory dysfunction and attention deficits.1 However, it is still unclear whether these symptoms are caused by chronic cerebral hypoperfusion or rather by a general structural damage of the brain tissue. In this still ongoing study, we assess the specific relation between one-sided cerebral blood flow (CBF) reductions and lateralized attention deficits.
Methods: 17 patients (70.5 ± 6.8 years) with one-sided (right: $n = 12$, left: $n = 5$), high-grade, asymptomatic ICAS (> 70%, NASCET criteria) and 26 healthy controls (70.7 ± 4.8 years) were enrolled in this prospective study. Participants underwent an MRI examination on a 3T Ingenia scanner (Philips), including MP-RAGE and FLAIR as well as perfusion MRI using pCASL.2 CBF-maps were normalized to MNI space and mean CBF was extracted within each hemisphere's watershed regions (CBF_right and CBF_left). An asymmetry index (AI) was calculated by the following formula: $AI = (CBF\_right)/(CBF\_right + CBF\_left)$. Furthermore, participants underwent a computer-based cognition test assessing spatial laterality of visual attention (w_λ) by computational fitting of attentional weights (w) of each side ($w_\lambda = (w\_left)/(w\_left + w\_right)$.3
Results: Patients had an increased absolute spatial laterality of visual attention ($|0.5\text{-}w_\lambda|$) with a trend to significance ($p = 0.088$). Concerning cerebral perfusion, patients showed a significantly higher absolute asymmetry ($|0.5\text{-}AI|$) than controls ($p = 0.001$). Most interestingly, AI

correlated significantly with w_λ in patients ($r = 0.538$, $p = 0.039$) whereas no significant association was observed in controls ($r = -0.122$, $p = 0.554$, see figure).

Conclusion: Unilateral cerebral hypoperfusion is significantly associated with contralateral attention deficits in patients with high-grade ICAS. Data indicate that chronic cerebral hypoperfusion as a potentially reversible condition impairs cognitive function.



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PS06-095

Poster Viewing Session VI

Quantification of GABA, glutamate and glutamine in a single measurement at 3T using GABA-optimized MEGA-PRESS

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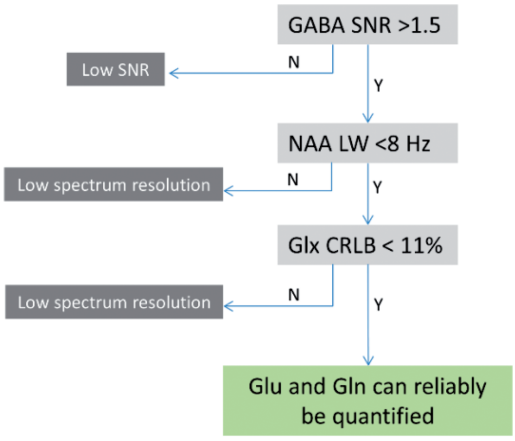
Abstract

Purpose: GABA and glutamate (Glu) are the major inhibitory and excitatory neurotransmitters in the brain and both are recycled through glutamine (Gln). All three metabolites can be measured by magnetic resonance spectroscopy in vivo, though GABA measurement requires an extra acquisition using the MEGA-PRESS editing sequence1. In GABA MEGA-PRESS, Glu and Gln co-edit with GABA providing the possibility of measuring the three in one acquisition2. Here the reliability of the composite Glu + Gln peak (Glx) estimation and the possibility of Glu and Gln separation in GABA MEGA-PRESS has been investigated. A sub-set of the data acquired in vivo was used to develop a framework which identifies MEGA-PRESS spectra in which Glu and Gln can be reliably estimated.

Method: Phantoms containing Glu, Gln, GABA and N-acetylaspartate at different concentrations were scanned using MEGA-PRESS optimized for GABA at 3T. 56 sets of spectra in 5 different brain regions were also acquired from 36 healthy volunteers. A peak by peak quality assessment was performed on all the data acquired in vivo.

Results: Using a GABA optimized MEGA-PRESS sequence, the Glu and Gln concentrations were accurately estimated across all phantoms with a linear relationship between measured and true concentration, $R^2 = 0.95$ for Glu and $R^2 = 0.91$ for Gln. A quality assessment framework was set based on the criteria necessary for a good GABA-edited MEGA PRESS spectra. The proposed quality assessment framework (Fig. 1.) judged 70% of the Glu and Gln estimations reliable. The data which were judged

unreliable also had a Glu/Gln ratio outside the physiological range.



[Fig.1]

Conclusion: Glu and Gln can be reliably quantified from GABA optimized MEGA-PRESS acquisitions. However this reliability should be controlled using quality assessment methods suggested in this work.

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PS06-096

Poster Viewing Session VI

Estimating subject numbers for study designs using functional GABA spectroscopy in human brain

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Abstract

Objective: Functional magnetic resonance spectroscopy (fMRS) is a research tool for understanding metabolic changes in the active brain. GABA has been studied with fMRS as a biomarker for investigations of the underlying biochemistry of brain function such as in motor cortical plasticity1 and visual simulation2. MEGA-PRESS has been the preferred pulse sequence for GABA measurements and has been shown to have repeatability and reliability3. However, low GABA concentration and long acquisition time makes its functional measurement challenging. Here we estimate the sample sizes and the necessary acquisition duration to detect concentration changes in GABA using MEGA-PRESS at 3T for common study designs in 7 brain regions (a) between-groups and (b) within-session measurements.

Method: 56 GABA spectra were acquired at 3T in seven brain regions in healthy volunteers at rest: anterior cingulate, left and central occipital cortex, left and right motor cortex, anterior temporal lobe and sensory motor cortex. We estimated between-groups, within-session and regional mean variability of GABA concentration.

Results: The Figure (a,b) illustrates mean and standard deviation (S.D.) of GABA across subjects for each region for increasing acquisition time. S.D. stabilizes after 4 min of acquisition in all regions. Power calculations (c,d) suggest that for example, detecting a 40% change in a 9 min acquisition, depending on the region of interest, requires from 10–15 subjects per group in a crossover design. Within-session analysis is ongoing.

Conclusion: fMRS of GABA can be successfully implemented in human brain; however three important factors of acquisition time, number of subjects and region of interest should be taken into consideration in order to have reliable results and potentially clinically be used in brain function disorders.

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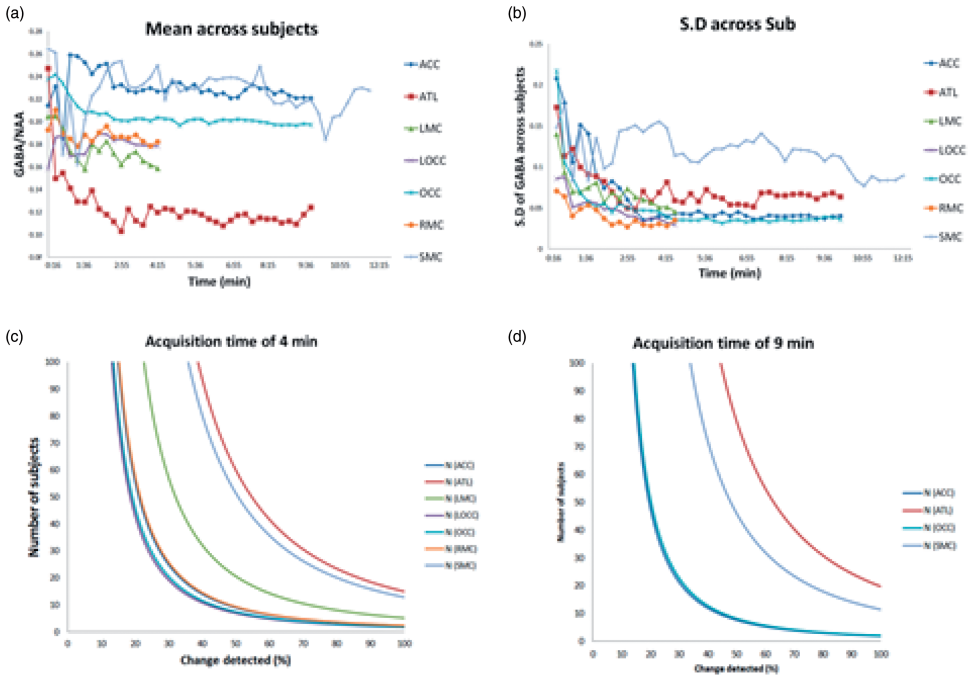

PS06-097

Poster Viewing Session VI

Brain astroglial reaction in mild hepatic encephalopathy: experimental evidence in the cirrhotic rat

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Abstract
Hepatic encephalopathy (HE) is a neuropsychiatric disorder that occurs in both acute and chronic liver failure. However, the pathomechanisms of the disease remains obscure. Neuopathological studies have demonstrated a primary gliopathy in humans as well as in animal models of chronic and acute liver failure. Here, we have investigated in an animal model of mild HE: the bile duct ligated rat (BDL) at the cirrhotic stage (4 weeks after surgery), the expression of the key marker of mature astrocytes; the glial fibrillary acidic protein (GFAP) in different brain areas such as: Substantia nigra pars compacta (SNc), Ventral tegmental area (VTA), hippocampus, dorsal striatum and brain cortex by means of immunohistochemistry. The immunohistochemical study showed, in BDL compared to the operated controls (shams), a diminished astrocyte reactivity corresponding to a loss of GFAP expression within SNc, VTA, hippocampus and dorsal striatum ($p < 0.05$), whereas in the brain cortex astrocytes appeared strongly immunoreactive with increased GFAP expression ($p < 0.05$) as compared to shams. Our finding demonstrated differential astroglial responses which depend to the specificity of the area investigated and its particular neuronal neighboring environment, and could have possible outcomes on the diverse neuronal functions especially those observed during the different episodes of hepatic encephalopathy.

PS06-098

Poster Viewing Session VI

Metabolite concentration changes during increase of the BOLD signal in the human visual cortex: a functional magnetic resonance spectroscopy study at 7T

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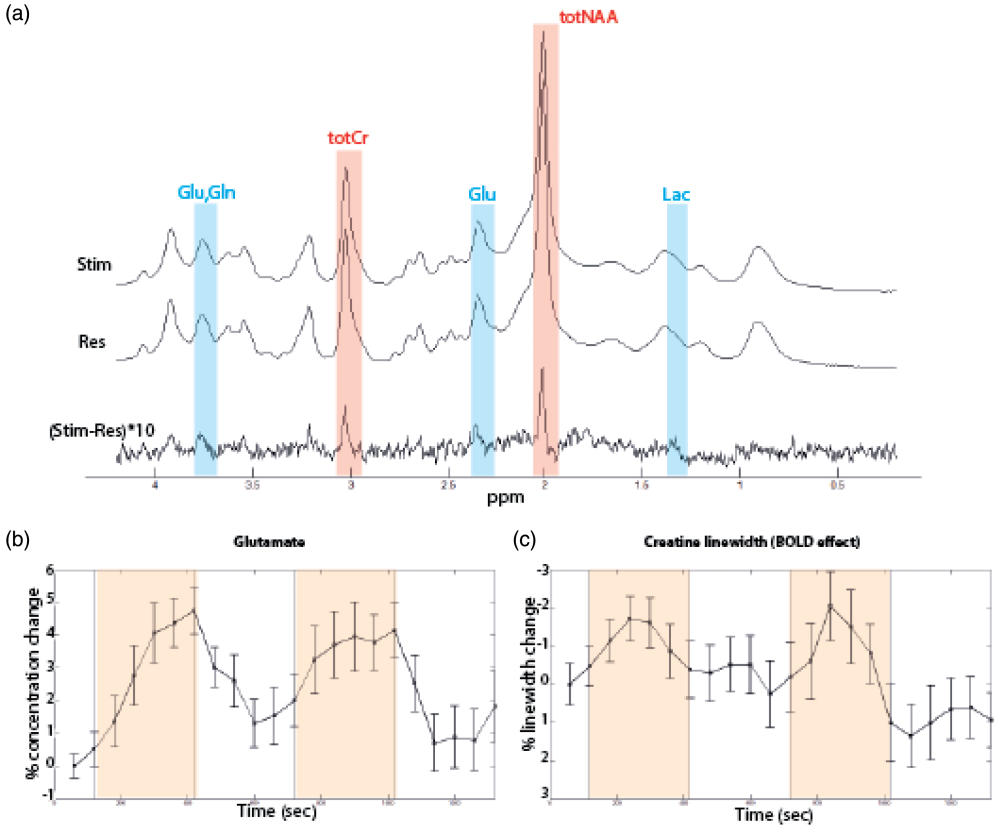
Abstract

Objective: Increase of neuronal activity requires changes in neurotransmitter cycling and energy support. These phenomena are observable in humans by functional magnetic resonance imaging (fMRI; BOLD changes) and spectroscopy (fMRS; metabolite changes). In this study, metabolite concentration changes in the visual cortex, occurring during an increase of the BOLD signal, were measured.

Methods: Height participants (19–24 years old) were scanned at 7T (Siemens) with a surface coil over the occipital area. A fMRI localizer was acquired to place the VOI in the region with highest activity. 1H-MR spectra were acquired using the semi-adiabatic SPECIAL sequence1 (TR/TE = 7500/16 ms, VOI = 18 × 18 × 18mm3, 88 × 2 scans) with the linewidth minimized with FAST(EST)MAP2. A visual stimulation was performed with a full screen radial checkerboard (STIM) and a gray screen (REST) for both the localizer (10 s STIM - 20 s REST x 12) and the fMRS (2 min REST and four alternate periods of 5 min STIM and REST; 22 min total). After B0 drift correction, the spectra were averaged and quantified with the LCModel3.

Results: A BOLD effect reflecting brain activation during STIM was observed by the change of linewidth of the creatine peak (Figure1A&C). Paired T-tests showed glutamate (0.26 μmol/g; $p < .05$ FDR-corrected) and lactate



(0.16 μmol/g; $p < .05$ uncorrected) concentration significant increases during brain activity (Figure 1A&B). Glutamate increased consistently until the end of STIM followed by a rapid return to baseline, while the linewidth change reached a maximum earlier followed by a return to baseline at the end of STIM.
[Figure1]
Conclusion: Activation of the visual cortex resulted in an increase of lactate and glutamate concentrations, as already observed previously, reflecting changes in neurotransmission and oxidative metabolic activities4.

References:

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2) Gruetter and Tkac, 2000
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4) Schaller et al., 2012

PS06-099

Poster Viewing Session VI

Effect of body position on cerebrovascular reactivity in healthy men and women

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Abstract
Cerebrovascular reactivity represents the ability to dilate the cerebral vessels to metabolic stimuli such as CO_2. However, it is unknown if reactivity is altered between supine, seated and standing positions and if the response is different between men and women. Five healthy men (range 18–47 years) and four healthy women (range 23–54 years) were asked to breathe room air, a hypercapnic mixture of 5% CO_2, 21% O_2, balance nitrogen, and to mildly hyperventilate (hypocapnia) for two minutes each (resulting in ~±10 mmHg change), while continuous measurements of blood pressure, heart rate, cerebral blood flow velocity of the middle cerebral artery, and end-tidal CO_2 were taken. Results show that the overall reactivity across both hypercapnia and hypocapnica ranges were not significantly different between the body positions ($p = 0.141$) or between the sexes ($p = 0.193$; Supine: men = 3.4 ± 0.6%/mmHg vs women = 2.9 ± 0.5%/mmHg; Seated: men = 2.7 ± 0.6%/mmHg vs women = 2.6 ± 0.2%/mmHg; Standing: men = 3.1 ± 0.4%/mmHg vs women = 2.7 ± 0.8%/mmHg). When examining the dilatory response during hypercapnia, there was no significant difference between the body positions ($p = 0.217$) or between the sexes ($p = 0.574$; Supine: men = 3.2 ± 1.5% vs women = 3.3 ± 1.3%; Seated: men = 2.4 ± 0.7% vs women = 3.0 ± 1.3%; Standing: men = 2.6 ± 0.8% vs women = 3.1 ± 0.9%). When measuring the vasoconstrictive response during hypocapnia, there was no significant difference across body positions ($p = 0.271$), but there was a trend towards significantly greater vasoconstriction in men compared to women ($p = 0.088$; Supine: men = 3.8 ± 1.0%/mmHg vs women = 2.9 ± 0.9%/mmHg; Seated: men = 3.3 ± 1.5%/mmHg vs women = 2.1 ± 1%/mmHg; Standing: men = 3.2 ± 0.4%/mmHg vs women = 2.3 ± 0.8%/mmHg). In addition, there was a large effect for this trend (partial eta squared = 0.359). Further participants are needed to assess whether there are differences in reactivity across the body positions or between the sexes. However, if the trend continues with additional participants, these results may indicate an enhanced vasoconstrictive response in men compared to women.

PS06-100

Poster Viewing Session VI

Postnatal development of neurovascular coupling

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Abstract
Objectives: Increases in local neural activity in the adult brain lead to increases in the delivery of oxygenated blood, a relationship termed neurovascular coupling. The resulting increases in oxygenation far exceed the oxygen used by the active tissue. Prior studies have shown a variety of hemodynamic responses in the newborn brain, ranging

from adult-like increases in oxygenation to decreases in oxygenation secondary to oxygen consumption and/or vasoconstriction effects. Earlier work from our lab in a rodent model demonstrated that hemodynamic responses to stimulation in newborns differ markedly from adult responses (Kozberg, 2013).

In this new study, we aimed to determine the neural activity underlying developmental differences in hemodynamics and define the resulting oxygenation dynamics (Kozberg, 2016).

Methods/Results: We developed a high-speed multispectral optical intrinsic signal and fluorescence imaging system to enable cortex-wide simultaneous imaging of neural activity and blood oxygenation in mice age postnatal day 7 - 13 as well as in adult mice, using GCaMP fluorescence as a marker of wide-field neural activity. We spatiotemporally mapped neural and hemodynamic activity (both spontaneous and stimulus-evoked) in awake and anesthetized conditions. Our novel imaging technique also enabled a detailed examination of the functional development of cortex-wide neural circuits.

Localized neural activity (both spontaneous and in response to stimulation) in early postnatal mice led to minimal increases in local blood flow. These hemodynamic responses were insufficient to meet the energy demands of activated brain regions, leading to relative hypoxias in active regions that were verified via metabolic imaging.

Conclusions: Minimal postnatal neurovascular coupling may be not only a consequence of immature neural and vascular networks, but also an important component of vascular patterning in the postnatal brain. Activity-dependent hypoxias may in fact direct vascular growth to frequently utilized areas. These findings have important implications for understanding normal and abnormal brain development.

PS06-101

Poster Viewing Session VI

Selective knockout of microglial Na^+/H^+ exchanger isoform 1 in mice does not reduce acute stroke brain injury but improves neuronal function recovery

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Abstract

Na^+/H^+ exchanger isoform-1 (NHE1) is ubiquitously expressed in all cell types in the central nervous system (CNS) and plays a role in ischemic brain damage. In this study, *CamKIICre*$^{+/-}$*;Nhe1*$^{flox/flox}$ and *Cx3cr1CreER*$^{+/-}$*; Nhe1*$^{flox/flox}$ mice were established to evaluate the roles of NHE1 in neurons and microglia in ischemic brain damage. In the case of *Cx3cr1CreER*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice, either corn oil (3.75 ml/kg body weight/day) or tamoxifen (Tam, 75 mg/kg/day, i.p., for 5 days) was given to mice at P30–34. Ischemic stroke was induced at P65–75 with 60 min of transient middle cerebral artery occlusion (tMCAO). Compared to *CamKIICre*$^{+/-}$ control mice, *CamKIICre*$^{+/-}$*; Nhe1*$^{flox/flox}$ mice showed a significant reduction in infarct volume at 48 h post-tMCAO, and improvements in neurological function 1–14 days after stroke ($p < 0.05$). In contrast, there were no decreases in ischemic infarct volume detected in the Tam-treated *Cx3cr1CreER*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice at 48 h after ischemic stroke; but these mice recovered significantly faster in their neurological function during 1–14 days after tMCAO ($p < 0.05$). They also exhibited a significantly higher survival rate during 1–14 days after ischemia. The flow cytometry analysis revealed that selective deletion of microglial NHE1 in the *Cx3cr1CreER*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice reduced $CD11b^+/CD45^{low\text{-}medium}$ microglial population in ischemic brains at 3 days post-ischemia, and increased populations of microglia with the Ym1 and CD206 anti-inflammatory phenotype, and decreased pro-inflammatory CD16/32 and CD86 expression. In comparison, selective deletion of neuronal NHE1 in the *CamKIICre*$^{+/-}$*;Nhe1*$^{flox/flox}$ mice showed no differences in the $CD11b^+/CD45^{low\text{-}medium}$ microglial population from the control mice during 1–14 days after ischemic stroke. These findings strongly suggest that microglial NHE1 and neuronal NHE1 play differential roles in acute ischemic brain injury formation and post-stroke recovery. Especially, microglia-mediated inflammation is involved in the brain tissue repair and functional plasticity.

This project is supported by NIH R01 NS048216 (D. Sun), AHA Postdoctoral Fellowship 17POST32440002 (S. Song).